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Form PTO-1390 (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
9013.43	
U.S. APPLICATION NO. (If known, see 37 CFR 1.5)	
10/069082	
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE
PCT/GB00/03159	16 August 2000
PRIORITY DATE CLAIMED	
18 August 1999	

TITLE OF INVENTION
Retrovirus Assay

APPLICANT(S) FOR DO/EO/US
Daniel GALBRAITH; Helena KELLY; Kenneth SMITH

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4)
7. ☐ Amendments to the claims of the International Application Under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report Under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4)
20. ☐ Other items or information: Written Opinion; International Search Report

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY DOCKET NO.
10/069082	PCT/GB00/03159	9013.43

21. ☐ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a) (1) - (5)):	CALCULATIONS	PTO USE ONLY
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO..... **$1040.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO..... **$890.00**		
International preliminary examination fee 37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... **$740.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... **$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)..... **$100.00**		
ENTER APPROPRIATE BASE FEE AMOUNT =	$1040.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	19 - 20 =	0	x **$18.00**	$	
Independent Claims	1 - 3 =	0	x **$84.00**	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$280.00**	$280.00	
TOTAL OF ABOVE CALCULATIONS =				$1320.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2			-	$	
SUBTOTAL =				$1320.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).			+	$	
TOTAL NATIONAL FEE =				$1320.00	
Fee for Recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property			+	$	
TOTAL FEES ENCLOSED =				$1320.00	
				Amount to be refunded:	$
				charged:	$

a. ☒ A check in the amount of $1320.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 50-0220 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☐ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-0220. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Kenneth D. Sibley, Reg. #31,665

Date: February 15, 2002

20792
PATENT TRADEMARK OFFICE

CERTIFICATE OF EXPRESS MAILING

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I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to: BOX PCT, Attn: DO/EO/US, Commissioner for Patents, Washington, DC 20231.

Vickie Diane Prior

Attorney Docket No. 9013.43

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	Galbraith, et al.
U.S. Serial No.:	10/069,082
Int'l Application No.:	PCT/GB00/03159
Int'l Filing Date:	August 26, 2000
For:	RETROVIRUS ASSAY

October 25, 2002

Commissioner for Patents
Washington, DC 20231

STATEMENT IN SUPPORT OF FILING A SEQUENCE LISTING UNDER 37 CFR § 1.821(f)

Sir:

Submitted herewith is a copy of the Sequence Listing in computer readable form. I hereby state that the content of the paper and computer readable copies of the Sequence listing are the same. I also hereby state as required by 37 CFR § 1.821(h) that the computer readable copy submitted concurrently herewith contains no new matter, nor does it go beyond the disclosure of the application as filed.

Respectfully submitted,

E. Michael Sajovec
Registration No. 31,793

Certificate of Mailing under 37 CFR 1.8

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, DC 20231, on October 25, 2002.

Vickie Diane Prior

Date of Signature: October 25, 2002

IN THE UNITED STATES DESIGNATED OFFICE (DO/US)

In re: Galbraith, et al.
Serial No. To Be Assigned
Filed: Concurrently Herewith
For: RETROVIRUS ASSAY

February 15, 2002

Box PCT
Attn: DO/US
Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT

Sir:

Prior to the examination of the above application, please amend the above-identified application as follows. If any extension of time for the accompanying response or submission is required, Applicant requests that this be considered a petition therefor. The Commissioner is hereby authorized to charge any additional fee, which may be required, or credit any refund, to our Deposit Account No. 50-0220.

In the Specification:

-- RELATED APPLICATIONS

The present application claims priority from International Application No. PCT/GB00/03159, filed on 16 August 2000, which in turn claims priority from British application 9919604.0, filed on 18 August 1999, the disclosures of which are hereby incorporated herein by reference in their entirety.--

In the Claims:

Please enter the following amended claims:

13 (amended). An antiserum specific to a polypeptide fragment in accordance with Claim 1.

14 (amended). A PoERV specific antibody or fragment thereof raised against a polypeptide fragment in accordance with Claim 1.

16 (amended). Use of a polypeptide fragment according to Claim 1 in detection of PoERV antibodies in a sample.

18 (amended). An assay kit for use in detection of PoERV antibodies in a sample, the kit comprising a polypeptide fragment in according with Claim 1.

19 (amended). Use of an antibody according to Claim 14, or a polypeptide fragment according to Claim 1 in therapy or diagnosis.

REMARKS

Claims 1-19 are presented for examination. The above claims have been amended to better conform to U.S. practice. Applicants respectfully request substantive examination on the merits. A copy of the claims showing the changes made is attached hereto as a "Version with Markings to Show Changes Made".

Respectfully submitted,

Kenneth D. Sibley
Registration No. 31,665

CERTIFICATE OF EXPRESS MAILING

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Vickie Diane Prior
Date of Signature: February 15, 2002

VERSION WITH MARKINGS TO SHOW CHANGES MADE

13 (amended). An antiserum specific to a polypeptide fragment in accordance with **[any preceding claim]** Claim 1.

14 (amended). A PoERV specific antibody or fragment thereof raised against a polypeptide fragment in accordance with **[any of]** Claim**[s]** 1 **[to 12]**.

16 (amended). Use of a polypeptide fragment according to **[any one of]** Claim**[s]** 1 **[to 12]** in detection of PoERV antibodies in a sample.

18 (amended). An assay kit for use in detection of PoERV antibodies in a sample, the kit comprising a polypeptide fragment in according with **[any of]** Claim**[s]** 1 **[to 12]**.

19 (amended). Use of an antibody according to Claim 14, or a polypeptide fragment according to **[any one of]** Claim**[s]** 1 **[to 12]** in therapy or diagnosis.

RETROVIRUS ASSAY

The present invention relates *inter alia* to porcine endogenous retrovirus (PoERV) fragments, in particular to *gag* and *env* fragments of PoERV. The invention relates further to use of such fragments in detection of PoERV or detection of exposure to PoERV. There is also provided antibodies to *gag* and *env* polynucleotides and polypeptides; kits for the detection of PoERV or exposure to PoERV; and *env* peptides and antisera specific for the various types of PoERV and use of such peptides and antisera in the detection of specific PoERV types.

Porcine endogenous retrovirus (PoERV) is an endogenous Gammaretrovirus present typically as a provirus found in several loci in the porcine genome. The proviral genome can be silent or is expressed. Expression of the virus was found to be associated with leukaemic pigs (Strandstrom et al, 1974) and some continuous porcine cell lines produce PoERV (Todaro et al, 1974). Virus from these cells has been shown to infect non-porcine cell-types including human cells (Galbraith et al, 1997; Patience et al, 1997). Three subgroups of PoERV have been described and are designated PoERV A, B and C dependent on the tropism of the virus and the related envelope gene structure (Onions et al, 1998). Only subtypes A and B have been shown to be capable of consistently infecting human cells in vitro. Subgroup C PoERV from mini-pigs has only been shown to infect one human cell line and this may reflect a low capacity for

infection of human cells. Since PoERV is expressed in pigs there is the potential for virus to be present in material prepared from pigs. Furthermore, as a consequence of xenotransplantation using porcine donor organs, there is the possibility that the endogenous virus will be expressed in vivo and be a potential risk of PoERV infection of the patient and the general population thereafter.

A number of different types of PoERV are known, based on their genetic makeup. Types designated PERV A, PERV B, and PoEV1 are described in International Patent Application WO97/40167, while types designated PERV MSL and Tsukuba are described in International Patent Application WO97/21836.

PoERV viruses comprise three genes: *gag, pol,* and *env,* generating GAG, POL and ENV polypeptides. It has been observed that the *gag* region of the genome appears to be substantially conserved among different viruses, as well as between PoERV virus types, while the *env* region contains both conserved and non-conserved regions, which non-conserved regions are observed to vary between viral types.

It is among the objects of the present invention to provide means whereby patients and/or samples may be monitored for viral infection. It is further among the objects of the present invention to provide means whereby the viral type may be determined.

According to one aspect of the present invention there is provided a PoERV polypeptide fragment, wherein said polypeptide fragment has PoERV specific antigenic or immunogenic activity. Antigenic or immunogenic activity is

to be understood as capable of eliciting a PoERV specific immune response when introduced into a normal mammalian host. For example, PoERV specific antibodies are produced as a consequence.

According to a further aspect of the present invention there is provided an antiserum specific to a PoERV polypeptide fragment as described above.

According to one aspect of the present invention, there is provided a fragment of a PoERV GAG polypeptide, wherein said fragment has PoERV specific antigenic or immunogenic activity. Such a fragment will be referred to hereinafter as a GAG peptide, it being understood that this is distinct from native GAG protein, and may comprise only a fragment thereof, provided the GAG peptide has antigenic activity. The consensus PoERV GAG polypeptide sequence is shown in Figure 1; in preferred embodiments of the invention, the GAG peptide may be selected from within this sequence.

According to a further aspect of the present invention, there is provided a fragment of a PoERV ENV polypeptide, wherein said fragment has PoERV specific antigenic or immunogenic activity. Such a fragment will be referred to hereinafter as an ENV peptide, it being understood that this is distinct from native ENV protein, and may comprise only a fragment thereof, provided the ENV peptide has antigenic activity. In one embodiment, the ENV peptide may be selected from within a conserved region of the various PoERV sequences, as illustrated in Figures 2

and 3. Conserved regions are those which comprise identical and/or highly conserved amino acid sequences in different PoERV virus types; wholly conserved amino acids are indicated in Figures 2 and 3 by an asterisk beneath the amino acid, with highly conserved amino acids being indicated by a colon. Alternatively, the ENV peptide may be selected from within the non-conserved regions of the various PoERV sequences of Figures 2 and 3, in which case the ENV peptide will be specific for a particular type of PoERV. Specific examples of non-conserved type-specific ENV peptides are shown as peptides D-H and J in Figure 3 In a third embodiment, the ENV peptide may comprise both a conserved and a non-conserved region of the PoERV ENV protein, from either adjacent or non-adjacent regions of the ENV protein. Such peptides may be considered useful in simultaneous detection of any PoERV virus and a specific viral type.

In a further aspect of the present invention, there is provided a fusion GAG/ENV peptide, which peptide comprises both GAG peptide sequences and ENV peptide sequences. Such peptide may be considered useful in simultaneous detection of any PoERV virus, by means of the GAG peptide, and a specific viral type, by means of the ENV peptide.

According to a yet further aspect of the present invention there is provided antibodies specific to either GAG or ENV peptides. The antibodies may be polyclonal or monoclonal. Such antibodies can include, but are not limited to, polyclonal antibodies, monoclonal antibodies

(mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, $F(ab')_2$ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. The Ig tails of such antibodies can be modified to reduce complement activation and Fc binding, (See, for example, European Patent No. 239400 B1, Aug. 3, 1994).

For the production of antibodies to a peptide, various host animals can be immunized by injection with a peptide, or a portion thereof. Such host animals can include but are not limited to rabbits, mice, and rats, to name but a few. Various adjuvants can be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Gurein) and *Corynebacterium parvum*.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen, such as target gene product, or an antigenic functional derivative thereof. For the production of polyclonal antibodies, host animals such as those described above, can be immunized by injection with a gene product supplemented with adjuvants as also described above.

Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, can be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to the hybridoma technique of Kohler and Milsrein, (1975, Nature 256:495-497; and US Pat. No. 4,376,110), the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today 4:72; Cole et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Anti-bodies And Cancer Therapy, Alan R. Liss, Inc., pp.77-96).

Such antibodies can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention can be cultivated in vitro or in vivo. Production of high titers of mAbs in vivo makes this the presently preferred method of production.

In addition, techniques for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454; U.S. Pat. No. 4,816,567) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable

region derived from a murine mAb and a human immunoglobulin constant region.

Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778: Bird, 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) and for making humanized monoclonal antibodies (U.S. Pat. No. 5,225,539) can be utilized to produce anti-differentially expressed or anti-pathway gene product antibodies.

Antibody fragments which recognize specific epitopes can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries can be constructed (Huse et al., 1989, Science, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Further aspects of the present invention provide methods of screening serum or tissue from humans or animal recipients of porcine tissue for exposure to PoERV. These methods include: use of antibodies to GAG or ENV peptides in the capture and/or detection of PoERV antigens; use of antibodies to GAG or ENV peptides in the detection of PoERV gene expression in virus infected cells by indirect immunofluorescence staining; the use of antibodies to GAG

or ENV peptides in the visualisation of PoERV virions in a sample by immuno-electron microscopy; use of GAG or ENV peptides in Western blotting for the detection of PoERV antibodies in samples from recipients of porcine-derived materials; and the use of GAG or ENV peptides in an enzyme-linked immunosorbent assay (ELISA) for the detection of PoERV antibodies.

Each of these methods may non-specifically detect any PoERV type (if GAG peptides, conserved ENV peptides or antibodies are used) or specific PoERV types, if type-specific non-conserved ENV peptides or antibodies are used.

The present invention also encompasses assay kits including GAG or ENV peptides or antibodies to such peptides, for use in the abovementioned assays. In preferred embodiments, the kits may further comprise any or all necessary preparative reagents, washing reagents, detection reagents and signal producing reagents commonly known in the art.

In all of these assays and methods, a number of distinct peptides or antibodies may be used, either sequentially or simultaneously, and differently labelled, in order to detect a number of different PoERV types in a single assay.

Diagnostic assays based upon the present invention may be used to determine the presence or absence of PoERV infection, and the PoERV type involved.

In an assay for the diagnosis of viral infection, there are basically three distinct approaches that can be adopted, involving the detection of viral nucleic acid, viral antigen or viral antibody respectively. Viral nucleic acid is generally regarded as the best indicator of the presence of the virus itself and would identify materials likely to be infectious. However, the detection of nucleic acid is not usually as straightforward as the detection of antigens or antibodies since the level of target can be very low. Viral antigen is used as a marker for the presence of virus and as an indicator of infectivity. Depending upon the virus, the amount of antigen present in a sample can be very low and difficult to detect. Antibody detection is relatively straightforward because, in effect, the host immune system is amplifying the response to an infection by producing large amounts of circulating antibody. The nature of the antibody response can often be clinically useful, for example IgM rather than IgG class antibodies are indicative of a recent infection, or the response to a particular viral antigen may be associated with clearance of the virus. Thus the exact approach adopted for the diagnosis of a viral infection depends upon the particular circumstances and the information sought. In the case of PoERV, a diagnostic assay may embody any one or a combination of these three approaches.

In an assay for the diagnosis of PoERV involving detection of viral antigen or antibody, the method may comprise contacting a test sample with a peptide of the present invention or a polyclonal or monoclonal antibody against the peptide and determining whether there is any antigen-antibody binding contained within the test sample. For this purpose, a test kit may be provided comprising a peptide, as defined herein, or a polyclonal or monoclonal antibody thereto and means for determining whether there is any binding with antibody or antigen respectively contained in the test sample to produce an immune complex. The test sample may be taken from any appropriate tissue or physiological fluid, such as blood (e.g. serum or plasma), saliva, urine, cerebrospinal fluid, sweat, tears or tissue exudate. If a physiological fluid is obtained, it may optionally be concentrated for any viral antigen or antibody present.

A variety of assay formats may be employed. The peptide can be used to capture selectively antibody against PoERV from solution, to label selectively the antibody already captured, or both to capture and label the antibody. In addition, the peptide may be used in a variety of homogeneous assay formats in which the antibody reactive with the peptide is detected in solution with no separation of phases.

The types of assay in which the peptide is used to capture antibody from solution involve immobilization of the peptide on to a solid surface. This surface should be

capable of being washed in some way. Examples of suitable surfaces include polymers of various types (moulded into microtitre wells; beads; dipsticks of various types; aspiration tips; electrodes; and optical devices), particles (for example latex; stabilized red blood cells; bacterial or fungal cells; spores; gold or other metallic or metal-containing sols; and proteinaceous colloids) with the usual size of the particle being from 0.02 to 5 microns, membranes (for example of nitrocellulose; paper; cellulose acetate; and high porosity/high surface area membranes of an organic or inorganic material).

The attachment of the peptide to the surface can be by passive adsorption from a solution of optimum composition which may include surfactants, solvents, salts and/or chaotropes; or by active chemical bonding. Active bonding may be through a variety of reactive or activatable functional groups which may be exposed on the surface (for example condensing agents; active acid esters, halides and anhydrides; amino, hydroxyl, or carboxyl groups; sulphydryl groups; carbonyl groups; diazo groups; or unsaturated groups). Optionally, the active bonding may be through a protein (itself attached to the surface passively or through active bonding), such as albumin or casein, to which the viral peptide may be chemically bonded by any of a variety of methods. The use of a protein in this way may confer advantages because of isoelectric point, charge, hydrophilicity or other physico-chemical property. The viral peptide may also be attached to the surface (usually

but not necessarily a membrane) following electrophoretic separation of a reaction mixture, such as immunoprecipitation.

After contacting the surface bearing the peptide with a test sample (in the presence of a blocking mixture if required), allowing time for reaction, and, where necessary, removing the excess of the sample by any of a variety of means, (such as washing, centrifugation, filtration, magnetism or capillary action) the captured antibody is detected by any means which will give a detectable signal. For example, this may be achieved by use of a labelled molecule or particle as described above which will react with the captured antibody or any molecule containing an epitope contained in the peptide. In one embodiment, it is preferred to add an anti-human IgG conjugated to horseradish peroxidase and then to detect the bound enzyme by reaction with a substrate to generate a colour.

The detectable signal may be produced by any means known in the art such as optical or radioactive or physico-chemical and may be provided directly by labelling the molecule or particle with, for example, a dye, radiolabel, fluorescent, luminescent, chemiluminescent, electroactive species, magnetically resonant species or fluorophore, or indirectly by labelling the molecule or particle with an enzyme itself capable of giving rise to a measurable change of any sort. Alternatively the detectable signal may be obtained using, for example, agglutination, or through a

diffraction or birefrigent effect if the surface is in the form of particles.

Assays in which a peptide itself is used to label an already captured antibody require some form of labelling of the peptide which will allow it to be detected. The labelling may be direct by chemically or passively attaching for example a radiolabel, magnetic resonant species, particle or enzyme label to the peptide; or indirect by attaching any form of label to a molecule which will itself react with the peptide. The chemistry of bonding a label to the peptide can be directly through a moiety already present in the peptide, such as an amino group, or through an intermediate moiety, such as a maleimide group. Capture of the antibody may be on any of the surfaces already mentioned in any reagent including passive or activated adsorption which will result in specific antibody or immune complexes being bound. In particular, capture of the antibody could be by anti-species or anti-immunoglobulin-sub-type, by rheumatoid factor, proteins A, G and the like, or by any molecule containing an epitope contained in the peptide.

The labelled peptide may be used in a competitive binding fashion in which its binding to any specific molecule on any of the surfaces exemplified above is blocked by antigen in the sample. Alternatively, it may be used in a non-competitive fashion in which antigen in the sample is bound specifically or non-specifically to any of the surfaces above and is also bound to a specific bi- or

poly-valent molecule (e.g. an antibody) with the remaining valencies being used to capture the labelled peptide.

Examples of the invention will now be described by way of illustration only, and with reference to the accompanying Figures, in which:

Figure 1 is the consensus amino acid sequence of the PoERV GAG protein; Figure 2 is a comparison of amino acid sequences of five different PoERV ENV proteins (PERV; PERV A, PERV B, PoEV 1; Galbraith et al., 1997: PERV MSL, Tsukuba; Fishman, 1997) and Figure 3 is a comparison of amino acid sequences of the variable region of five different PoERV ENV proteins, showing the six different ENV peptides (peptides D-H and J) referred to in the following examples.

METHODS

Preparation of PoERV virions.

Human 293 cells (American Type Culture Collection [ATCC] # CRL1573) and Raji cells (ATCC # CCL 86) were infected with PoERV by exposure to polybrene (Sigma-Aldrich Co. Ltd.) and continued incubation with cell-free filtered supernatant from PK-15 (ATCC # CCL 33) cells previously shown to be infected with all three subgroups of PoERV. The 293 cells allow replication of type B PoERV (POEV-1). The 293 cells were shown to be infected after passage by measurement of the reverse transcriptase activity of the cell supernatant and by a PoERV GAG-specific Polymerase Chain Reaction (PCR). The resulting virus particles were isolated from the cell line supernatant as follows.

Supernatant from exponentially growing cells was layered onto a 20/40% (w/v) discontinuous sucrose density gradient and centrifuged at 100,000 g for 150 min. The viral material at the sucrose interface was harvested, and viral particles pelleted by further ultracentrifugation at 100,000g for 60 min, followed by resuspension in DMEM (Life Technologies Ltd., UK).

Control retroviruses

To provide retroviral controls for cross reactivity with PoERV GAG and ENV, Squirrel monkey retrovirus, Murine leukaemia virus, Maedi-Visna virus and Equine infectious anemia virus virions were prepared from the appropriate infected cell line as described by Shepherd and Smith (1999).

Selection and preparation of GAG peptides

Peptides from the GAG protein can provide a capture antigen and a means to generate positive control antisera. The antisera can be directed against conserved polypeptides present in the PoERV virion core likely to induce an immune response in recipients of the virus. These reagents would be useful diagnostic tools for immunosurveillance of recipients of porcine material or tissues for exposure to PoERV. Therefore, peptides encompassing potential antigenic regions of PoERV GAG were selected from the translated amino-acids derived from the sequence of the gag region of PoERV based on three criteria; hydrophilicity,

potential ß-turns and K, D, R and E charged residues. The regions were identified using Hopp and Woods hydropathicity (1981) scale and Kyte and Doolittle (1982) hydrophobicity scale.

For GAG two peptides were identified as potential antigens. Peptide 1 was from the C-terminus of p30-GAG at residue 437-451 of the polypeptide, nucleotides 1896-1940 of the *gag* open reading frame (ORF). Peptide 2 was from the start of the p10 segment of the GAG polypeptide at residue 502-515 of the polypeptide, nucleotides 2091- 2132 of the *gag* ORF. The peptides shown below were chemically synthesised by Genosys Biotechnologies Inc.

Peptide 1: (C)REERRDRRQEKNLTK

Peptide 2: (W)ARNCPKKGNKGPKS

The bracketed amino-acid is not in sequence - 5' position is from next residue (R).

These peptides, and the GAG consensus polypeptide sequence, are shown in Figure 1.

A BLAST search (Altschui et al., 1997) of the non-redundant GenBank coding sequences with GAG peptide 1 showed homology with seven sequences all from the *gag* ORF. Of the seven, three were with PoERV sequences with accessions gi 3116446 (100% match), emb CAA7651 (100% match), gi 3116442 (86% match). The remainder were against the closely related Gibbon ape leukaemia virus (gi 3033415, 92% match) and Simian sarcoma virus (sp PO3330, 86% match).

The remaining two sequences were from murine viruses, including a virus from *Rattus norvegicus* (emb CAA24514; 92% match), and one against *Mus dunni* endogenous retrovirus (gi 3309124, 93% match). A similar BLAST search with GAG peptide 2 showed 100 - 99% homology with only the PoERV sequences listed above.

Selection and Preparation of PoERV ENV peptides

We have previously identified and derived the nucleotide sequence of a unique PoERV type capable of infecting human cells (Galbraith, 1997). Furthermore, it has been shown that the amino acid sequence of the ENV region of various PoERV types contains both conserved and non-conserved regions (Galbraith, 1997; Figure 2). In order to exploit these differences to produce immunological reagents to allow the identification of the type of PoERV giving rise to an immunological reaction in a patient, PoERV-type-specific ENV peptides and antisera were generated.

Six peptides, D-H and J, were identified as potential antigens. The peptides are shown below and their position in the *env* ORF of the various PoERV types is shown in Figure 3. The peptides were chemically synthesised by the University of Glasgow Veterinary Pathology Department.

Peptide D: TSLRPDITQPPSNSTT

Peptide E: KGKQENIQKWINGMS

Peptide F: RKTGKYSKVDKWYELGNS

Peptide G: NTVLTGQRPPTQ

Peptide H: GHGRWKDWQQRVQKDVRNKQIS

Peptide J: IQEQRPSPNPSDYNTT

The amino acid residues of all peptides are identified by the standard one letter abbreviations.

Preparation of recombinant PoERV p30-GAG and ENV polypeptides

In addition to the peptide reagents more general PoERV p30-GAG and an abbreviated ENV polypeptides were designed and produced for use as capture antigens and to produce anti-polypeptide sera. The required polypeptide portions of the *gag* and *env* genes were produced by PCR amplification, molecularly cloned into a prokaryotic expression vector and expressed as described below using standard techniques (Maniatis et al, 1982).

PoERV p30-GAG

A fragment encompassing the p30 region of the *gag* ORF from nucleotide 1173-1949 of the PoERV genome (Galbraith et al, 1997; Gene Bank Accession # A66553) was amplified by PCR from cDNA generated from PK15 mRNA using ligation independent cloning oligonucleotide primers (pET-32 Ek/LIC cloning and expression vector; Novagen Inc. Catalogue # 69076-3). The oligonucleotides were:

p30 forward 5' GAC GAC GAC AAG **CTG CGC ACC TAT GGC C** 3'

p30 reverse 5' GAG GAG AAG CCC GGG **TCT AGG CCA AGA TCT TAG TCA AAT TCT TCT C** 3'

The nucleotides in bold are viral specific.

The PCR conditions were 30 cycles of 95°C for 1 min, 58°C for 1 min and 72°C for 1 min. The resulting 776 base pair fragment was molecularly cloned into the appropriate LIC site of the pET-32 LIC vector following the manufacturer's instructions (Novagen Inc. 69076-3 instruction manual), transfected into competent Novoblue™ *Escherichia coli* cells and plated on solid LB medium containing ampicillin. The transformed colonies were selected by resistance to ampicillin.

PoERV ENV

A fragment encompassing the region of the *env* ORF from nucleotide 5616- 6304 of the PoERV genome (Galbraith et al,1997; Gene Bank Accession # A66553) was amplified by PCR from cDNA generated from PK15 and PoERV-infected 293 cells (PoERV B) mRNA using ligation independent cloning oligonucleotide primers (pET-32 Ek/LIC cloning and expression vector; Novagen Inc. Catalogue # 69076-3). The oligonucleotides were:

env forward 5' GAC GAC GAC AAG **ATC CAT GCA TCC CAC GTT** 3'

env reverse 5' GAG GAG AAG CCC GGT **CTC TAT CCT AAG GCG** 3'

The nucleotides in bold are viral specific.

The PCR conditions were 30 cycles of 95°C for 1 min, 60°C for 1 min and 72°C for 1 min. The resulting 688 base pair fragment was molecularly cloned into the appropriate LIC site of the pET-32 LIC vector following the manufacturer's instructions as described above.

For expression from the T7 promoter the recombinants are required to be transferred to a host with T7 polymerase activity. To this end plasmid DNA was isolated from the ampicillin resistant Novoblue™ clones carrying the *gag* or *env* fragment in the correct orientation for expression as determined by restriction endonuclease mapping. The plasmid DNAs were each transfected into competent *E. coli* AD494 (DE3) *trx B-* .

For screening for the production of recombinant protein, two ml cultures of *E. coli* transformed with either of the two expression constructs were grown with shaking at 37°C to late log phase ($O.D._{600nm}$ of approximately 0.6) and induced by the addition of Isopropylthio-beta-galactoside (IPTG) to 0.1 mM. Induced cultures were then incubated for a further 2 h after which the bacteria were collected by centrifugation. The bacterial pellet was lysed by boiling in SDS-PAGE sample buffer and the protein profile of the induced bacteria was analysed on a 12% acrylamide gel

followed by staining with coomasie brilliant blue dye. (Laemmli, 1970; Gallagher, 1997).

Large scale preparations of purified GAG and ENV polypeptides were made according to the manufacturer's instructions (Novagen Inc. Catalogue # 69076-3).

Preparation of antisera to whole virions, peptides and recombinant P30-GAG and ENV antigens.

For GAG peptide 1 and peptide 2, the peptides were conjugated with keyhole limpet hemacyanin carrier protein and each of two rabbits was inoculated six times at fourteen day intervals. The animals were bled out at day seventy seven after the first inoculation. The p30-GAG polypeptide was inoculated three times at fourteen day intervals into a rabbit. The animal was bled out at day seventy seven.

For ENV peptides D-H and J, the peptides were conjugated with keyhole limpet hemacyanin carrier protein and one sheep was inoculated three times at twenty eight day intervals. The ENV polypeptide was inoculated three times at fourteen day intervals into a rabbit.

Virions purified from PK15 cells were inoculated three times at fourteen day intervals into each of two guinea pigs.

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Virions purified from PK15 cells were inoculated three times at fourteen day intervals into each of two guinea pigs.

Indirect immunofluorescence staining

To test the specificity of the p30-GAG antisera the method outlined by Riggs (1989) was used. PoERV-infected Raji cells and uninfected control Raji cells were fixed and tested for indirect immunofluorescence with anti p30-GAG using a fluorescein isothiocyanate (FITC) labelled anti-rabbit detector antibody. The cells were examined by fluorescence microscopy.

Preparation of Western blot membranes

Recombinant p30-GAG polypeptide and ENV polypeptide were prepared, harvested and purified from an *E.coli* vector. The recombinant proteins were tested to determine an appropriate dilution of protein which yielded a positive result in the immunoassay. In addition, extracts from PoERV-infected 293 cells, PoERV-infected Raji cells or purified PoERV virions were used as antigens. To obtain specific and reproducible Western blot assays, a number of parameters were required to be optimised for each assay, such as: Primary antibody dilution, incubation time, incubation temperature, secondary antibody dilution, incubation time, incubation temperature, washing buffers, blocking/dilution buffers, developing reagents. Recombinant polypeptides were added to nine wells of a ten lane 12% Tris/glycine acrylamide gel. Molecular weight markers were added to the first lane. The samples were electrophoresed and the gel electroblotted to a poly vinylidene fluoride (PVDF) membrane. (Gallagher et al,

1997). The membrane was cut into strips each strip containing one lane of recombinant protein. These strips were used as the basis of the assay.

Preparation dilutions of antisera

Samples were prepared in a Class 2 safety cabinet or other clean environments.

A typical negative control was prepared by making up to a 1:200 dilution of normal sera in blocking reagent.

A typical positive control was prepared by making a 1: 500, 1: 1000 or greater dilution of anti-PoERV p30-GAG polypeptide, peptide serum or anti - recombinant ENV serum.

A typical test serum was prepared by making up to a 1:200 dilution of sera.

Preparation of Western blotting membranes and PoERV antibody detection.

To block non-specific binding sites membrane strips each were placed in a 15 ml centrifuge tube and 2 ml blocking reagent (2.5 g skimmed dried milk in 50 ml PBS/ 0.5% v/v Tween-20™) added. The strips were placed on a rotary shaker such that the strip moved slightly on each revolution and were incubated for 30 min at ambient

temperature. The blocking reagent was removed and replaced with 5-10 µl of the diluted serum. The membrane was incubated with shaking for 1 h at ambient temperature. To stop incubation the strip was removed from diluted serum and placed into PBS/ Tween-20™ and washed with three changes of PBS/ Tween-20™ at ambient temperature with shaking.

The appropriate species specific secondary antiserum conjugated to alkaline phosphatase was used as detector e.g. if human serum was being tested, an anti-human IgG alkaline phosphatase (AP) conjugate was used. The p30-GAG positive control required anti-rabbit IgG AP conjugate for detection and the anti ENV required anti-sheep IgG AP conjugate. The detection was done as follows; each strip was placed in an unused 15 ml centrifuge tube, 2 ml of 1:1000 dilution of secondary sera in blocking reagent was added and incubated with shaking at ambient temperature for 1 h. The strip was removed from the centrifuge tube, placed in PBS/Tween-20™ and washed with 3 changes of PBS/Tween-20™, at ambient temperature with shaking. The strips were then put into a 15 ml centrifuge tube and 2 ml of bromochlorindoyl phosphate/ nitroblue tetrazolium (BCIP/NBT, Sigma-Aldrich Co. Ltd.) solution was added to each tube. The strips were shaken gently and allowed to develop for 5 min. The reaction was stopped by rinsing the membrane strip in purified water and the strips were removed from the water and allowed to air dry.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

Antigen coating of microtiter plates

50 µl of antigen (either recombinant p30-GAG, extracts from PoERV-infected 293 cells or purified PoERV B virions) were diluted to the required concentration in carbonate-bicarbonate coating buffer (Sigma-Aldrich Co. Ltd.) was added to wells of a 96 well, flat bottomed microtiter ELISA plate (Dynex Immulon 2). Some wells contained carbonate-bicarbonate coating buffer only and some were left blank to act as controls for non-specific binding. The plate was covered with a plate seal and incubated at 4°C for approximately 16 h. The unbound antigen and coating solution were then removed from the wells with a pipette and washed three times with PBS / 0.05% Tween-20™. Any remaining PBS / 0.05% Tween-20™ was removed by blotting on a tissue.

Blocking of microtiter plates

50 µl of fresh blocking buffer (5% (w/v) skimmed milk /PBS / 0.05% Tween-20™) was added to each antigen coated well and control well. The plate was covered with a plate seal and incubated at ambient temperature in an humidified chamber for 1 h. The blocking buffer was removed and the plate wells washed three times with PBS / 0.05% Tween-20™ and any remaining PBS / 0.05% Tween-20™ was removed by blotting on a tissue.

Incubation with primary antibody

50 µl of negative control sera, test sera, and positive control sera at the experimental dilution were added to the antigen coated wells and the plates covered with a plate seal and incubated at ambient temperature for approximately 1 h in an humidified chamber. Following incubation all sera were removed using a pipette and the plate wells washed three times with PBS / 0.05% Tween-20™, any remaining PBS / 0.05% Tween-20™ was removed by blotting on a tissue.

Incubation and development with peroxidase-conjugated secondary antibody

50 µl of a 1:500 dilution of species specific peroxidase conjugated secondary antibody in blocking buffer was added to each antigen coated well. For human serum an anti-human IgG peroxidase conjugate was used. The p30-GAG positive control required anti-rabbit IgG peroxidase conjugate for detection and the anti ENV required anti-sheep IgG peroxidase conjugate. The plates were covered with a plate seal and incubated at ambient temperature for approximately 1 h in an humidified chamber. Following incubation the conjugate was removed using a pipette and the plate wells washed three times with PBS / 0.05% Tween-20™. Any remaining PBS / 0.05% Tween-20™ was removed by blotting on a tissue.

The substrate was prepared as follows: one O-phenylenediamine tablet (Sigma-Aldrich Co. Ltd.) and one urea/H_2O_2 tablet were dissolved in 20 ml of purified water.

An aliquot of 50 µl of substrate was added to each well and the plate incubated at ambient temperature in the dark for 30 min. The reaction was then stopped by adding 50 µl of 3N HCl or 3 M H_2SO_4 to each well. The colour development in the wells was measured at 490 nm using a Dynex MRX microplate reader.

For alkaline phosphatase conjugated secondary antibodies the substrate used was p-Nitrophenyl phosphate (pNPP; Sigma-Aldrich Co. Ltd.) and the plates were read at 405 nm.

Electron Microscopy

Negative stain electron microscopy (NSEM) (Doane, 1980) was used to identify the presence of PoERV virions. Supernatant from PoERV-infected pK15 cells was layered onto a 20/40% (w/v) discontinuous sucrose density gradient and centrifuged at 100,000 g for 150 min. The viral material at the sucrose interface was harvested, and viral particles pelleted by further ultracentrifugation at 100,000g for 60 min, followed by resuspension in DMEM (Life Technologies Ltd.). The sample was then applied to pioloform-coated copper 300 mesh EM grids and allowed to air dry. Grids were fixed with 2.5% glutaraldehyde (Agar Scientific), stained with 5% uranyl acetate (Agar Scientific) and allowed to air dry. Grids were examined on a Philips EM-400 transmission electron microscope.

Immuno- Electron Microscopy

The immunostaining was done following the method of Marshall et al (1992). Briefly, NSEM samples were applied to pioloform-coated nickel 300 mesh EM grids and allowed to air dry. Grids were fixed with modified immunofix, post fixed with 0.5 M NH_4Cl, then incubated with 2% bovine serum albumin (Sigma-Aldrich Co. Ltd.). Samples were then incubated with rabbit anti-PoERV (rabbits immunised with whole PoERV) or rabbit anti-PoERV p30-GAG antibody, washed in modified immunobuffer followed by incubation with anti-rabbit IgG gold conjugate (Sigma-Aldrich Co. Ltd.). Grids were stained with 5% uranyl acetate, and allowed to air dry. Samples were visualised on a Philips EM-400 transmission electron microscope.

EXAMPLE ONE

Indirect immunofluorescence staining of PoERV-infected cells.

Viral specific fluorescence was observed in PoERV-infected Raji cells using the anti p30-GAG antiserum. No immunofluorescence was seen with the negative control Raji cells.

EXAMPLE TWO

Western Blotting for Antibodies to PoERV GAG

Anti-GAG peptide 1 antisera and sera from rabbits inoculated with the recombinant p30-GAG polypeptide detected the expected protein of approximately 30kd in extracts of PoERV-infected 293 or PoERV-infected Raji cells, purified PoERV virions and recombinant p30-GAG. The PoERV antibody could be detected at a dilution of 1:1000.

No band of equivalent size to the GAG 30 kd polypeptide was detected in uninfected control cells.

No band of equivalent size to the GAG 30 kd polypeptide was detected against the following purified retroviruses:

Squirrel monkey retrovirus
Murine leukaemia virus
Maedi-Visna virus
Equine infectious anemia virus

Therefore, the positive control antisera were specific for PoERV.

Determination of assay specificity using Serum Panels

Normal human sera

On testing of 90 normal serum samples from healthy individuals, whose blood was taken for occupational health

reasons, no PoERV reactive antibody was detected in any of the sera.

Normal primate sera

On testing 42 normal serum samples from healthy primates no PoERV reactive antibody was detected. There was no cross-reactivity with sera from normal primates.

Cardiac transplant patient sera

On testing 20 serum samples from individuals who had received a cardiac transplant in the preceding 36 months no PoERV reactive antibody was detected. There was no cross-reactivity with sera from patients who had been immunosupressed.

HIV positive sera

On testing 13 serum samples from individuals positive for the presence of antibody to HIV no PoERV reactive antibody was detected. There was no cross-reactivity with sera from patients infected with a human Lentivirus.

HTLV positive sera

On testing 10 serum samples from individuals positive for the presence of antibody to HTLV-1 virus no PoERV reactive antibody was detected. There was no cross-reactivity with sera from patients infected with a human Gammaretrovirus.

Butchers with acute lymphoblastic leukaemia (ALL)

On testing 3 serum samples from butchers with ALL no PoERV reactive antibody was detected. There was no cross-reactivity with sera from patients with assumed prolonged exposure to PoERV and PoERV antigens with a leukaemic disorder.

EXAMPLE THREE

ELISA p30-GAG

A titration of p30-GAG antigen to anti-p30-GAG antisera gave a significant signal at 1:250600 dilution antigen to 1:32000 dilution of antisera. A similar titration of antisera against PoERV virions gave a significant signal at a 1:3200 dilution of both antigen and antisera.

Normal human sera

On testing of five normal serum samples from healthy individuals whose blood was taken for occupational health reasons, no significant signal was detected in any of the sera against recombinant p30-GAG.

EXAMPLE FOUR

Detection and Visualisation of PoERV Virions by Immuno-Electron Microscopy

Examination of PoERV virion preparations by negative stain revealed particles showing the characteristic size and structure of a Gammaretrovirus of approximately 90-120 nm with a dark inner core and double membraneous outer region.

The particles bound immuno-gold labeled anti p30-GAG antiserum indicating that the antiserum could be used to visualise PoERV virions by immuno-electron microscopy.

EXAMPLE FIVE

ELISA ENV

A titration of antisera raised against ENV peptides D, E, F, G, H, J. to purified PoERV B virions gave a significant signal indicating that the ENV peptides produced a virus-specific reaction in the animals. Peptides D and F, both from PoERV B (POEV1; Figure 2; Galbraith et al, 1997) gave the highest signal.

EXAMPLE SIX

Western Blotting for Antibodies to PoERV ENV

Antisera from guinea pigs inoculated with whole purified PoERV virions from PK15 cells detected the expected recombinant ENV protein of approximately 24 kD in extracts of *E.coli* expressing the *env* construct.

No band of equivalent size to the ENV 24 kD polypeptide was detected on *E.coli* control cells without the expresion construct.

REFERENCES

Altschui SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W and Lipman DJ (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research, 25: 3389-3402.

Doane FW . (1980). Virus morphology as an aid for rapid diagnosis. Yale Journal of Biological Medicine, 53, 19-25.

Ekins RP. (1997). Immunoassay design and optimisation. In Principles and Practice of Immunoassay Second Edition. Ed. CP Price and DJ Newman. Macmillan Reference, Ltd. London.

Fishman JA. (1997). Patent Application WO97/21836.

Galbraith DN. Haworth C, Lees GM, and Smith KT. (1997). Patent Application WO97/40167.

Gallagher S. (1997). One-dimensional SDS gel electrophoresis of proteins. In Current Protocols in Molecular Biology. 10.2.2-10.2.35. John Wiley & Sons, Inc. USA.

Gallagher S, Winston SE, Fuller SA and Hurrell JGR. (1997). Immunoblotting and immunodetection. In Current Protocols in Molecular Biology. 10.8.1-10.8.21. John Wiley & Sons, Inc. USA.

Hopp TP and Woods KR. (1981). Prediction of protein antigenic determinants from amino acid sequences. Proceeding of the National Academy of Sciences USA, 78, 3824-3828.

Kyte J and Doolittle RF. (1982). A simple method for displaying the hydropathic character of a protein. Journal of Molecular Biology, 157, 105-132.

Laemmli UK. 1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680-685.

Maniatis T, Fritsch EF and Sambrook J. (1982). Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Marshall GE, Kontas AG, Reid GG, Edwards JG and Lee WR. (1992). Type IV collagen and laminin in Bruch's membrane and basal linear deposit in the human macula. British Journal of Ophthalmology, 76, 607-614.

Onions D, Hart D, Mahoney C, Galbraith D and Smith K. (1998). Endogenous retroviruses and the safety of porcine xenotransplantation. Trends in Microbiology, 6, 430-431.

Patience C, Takeuchi Y and Weiss R. (1997). Infection of human cells by an endogenous virus of pigs. Nature

Medicine, 3, 282-286.

Riggs JL. (1989). Immunofluorescence staining. In Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections Sixth Edition. Ed. NJ Schmidt and RW Emmons. 123-133. American Public Health Association Inc. Washington DC.

Strandstrom H, Veijalainen P, Moennig V, Hunsmann G, Schwarz H and Schafer W. (1974). C-type particles produced by a permanent cell line from a leukemic pig. 1. Origin and properties of the host cells and some evidence for the occurrence of C-type particles. Virology, 57, 175-178.

Shepherd AJ and Smith KT. (1999). Viral evaluation of animal cell lines used in biotechnology. In Methods in Biotechnology, Vol. 8: Animal Cell Biotechnology. Ed. N Jenkins. Humana Press, Inc. Totwa New Jersey.

Todaro GJ, Benveniste RE, Lieber MM, and Sher CJ. (1974). Characterisation of a type C virus released from the porcine cell line PK (15). Virology, 58, 65-74.

CLAIMS

1. A porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment has PoERV specific antigenic or immunogenic activity.

2. A polypeptide fragment according to claim 1, wherein said polypeptide fragment comprises a fragment of a PoERV GAG polypeptide.

3. A polypeptide fragment according to claim 2, wherein said polypeptide fragment comprises a fragment of a polypeptide sequence as shown in Figure 1.

4. A polypeptide fragment according to claim 3, wherein said polypeptide fragment comprises the amino acid sequence REERRDRRQEKNLTK.

5. A polypeptide fragment according to claim 3, wherein said polypeptide fragment comprises the amino acid sequence ARNCPKKGNKGPKV.

6. A polypeptide fragment according to claim 1, wherein said polypeptide fragment comprises a fragment of a PoERV ENV polypeptide.

7. A polypeptide fragment according to claim 6 wherein said polypeptide fragment comprises a fragment of a polypeptide sequence as shown in Figures 2 or 3.

8. A polypeptide fragment according to claim 7 wherein said polypeptide fragment comprises a fragment from within a conserved region of the sequences shown in Figures 2 and 3.

9. A polypeptide fragment according to claim 7 wherein said polypeptide fragment comprises a fragment from within a non-conserved region of the sequences shown in Figures 2 and 3.

10. A polypeptide fragment according to claim 9 wherein said polypeptide fragment comprises an amino acid sequence selected from the sequences of peptides D, E, F, G, H and J as shown in Figure 3.

11. A polypeptide fragment according to claim 7 wherein said polypeptide fragment comprises a fragment from within a conserved region of the sequences shown in Figures 2 and 3, and a fragment from within a non-conserved region of the sequences shown in Figures 2 and 3.

12. A polypeptide fragment according to claim 1, wherein said polypeptide fragment comprises a fragment of a PoERV GAG polypeptide and a fragment of a PoERV ENV polypeptide.

13. An antiserum specific to a polypeptide fragment in accordance with any preceding claim.

14. A PoERV specific antibody or fragment thereof raised against a polypeptide fragment in accordance with any of claims 1 to 12.

15. Use of an antibody according to claim 14 in the detection of PoERV in a sample.

16. Use of a polypeptide fragment according to any one of claims 1 to 12 in the detection of PoERV antibodies in a sample.

17. An assay kit for use in detection of PoERV in a sample, the kit comprising an antibody in accordance with claim 14.

18. An assay kit for use in detection of PoERV antibodies in a sample, the kit comprising a polypeptide fragment in accordance with any of claims 1 to 12.

19. Use of an antibody according to claim 14, or a polypeptide fragment according to any one of claims 1 to 12 in therapy or diagnosis.

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(54) Title: RETROVIRUS ASSAY

PoERV *GAG* POLYPEPTIDE SHOWING POSITIONS OF PEPTIDES

MGQTVTTPLSLTLDHWTEVRSRAHNLSVQVKKGPWQTFCASEWPTFDVGWPSEGTFNSEIILAVKAIIFQ
TGPGSHPDQEPYILTWQDLAEDPPPWVKPWLNKPRKPGPRILALGERMKHSAEKVEPSSSYLPRDRGAAD
LAGTPTCSPTPLSSTGCCEGTSAPPGAPVVEGPAAGTRSRRGATPERTDEIAILPLRTYGPPMPGGQLQP
LQYWPFSSADLYNWKTNMPPFSEDPQRLTGLVESLMFSHQPTWDDCQQLLQTLFTTEERRILLEARKNV
PGADGRPTQLQNEIDMGFPLTRPGWDYNTAEGRESLKLYRQALVAGLRGASRRPTNLAKVREVMQGPNEP
PSVFLERLMEAFRRFTPFDPTSEAQKASVALAFIGQSALDIRKKLQRLEGLQEAELRDLVREAEKVYYRR
ETEEEKEQRKEKEREEreerdrrqeknltkILLAAVVEGKSSRERERDFRKIRSGPRQSGNLGNRTPLDK
DCCAYCKEKGHWarncpkkgnkgpkvLALEEDKD

The *gag* peptides 1 and 2 are shown in lower case bold. Peptide 1: REERRDRRQEKNLTK; Peptide 2: ARNCPKKGNKGPKV.

(57) Abstract: The present invention relates to polypeptide fragments derived from porcine endogenous retrovirus (PoERV) GAG and ENV polypeptides, and to their use in detection of PoERV antibodies in a test sample. Also provided are antibodies to GAG and ENV polypeptides, which may be used to detect PoERV in a sample. Polypeptide sequences are provided which are common to several strains of PoERV, as are sequences specific to a single PoERV strain.

MGQTVTTPLSLTLDHWTEVRSRAHNLSVQVKKGPWQTFCASEWPTFDVGWPSEGTFNSEIILAVKAIIFQ
TGPGSHPDQEPYILTWQDLAEDPPPWVKPWLNKPRKPGPRILALGEKNKHSAEKVEPSSSYLPRDRGAAD
LAGTPTCSPTPLSSTGCCEGTSAPPGAPVVEGPAAGTRSRRGATPERTDEIAILPLRTYGPPMPGGQLQP
LQYWPFSSADLYNWKTNHPPFSEDPQRLTGLVESLMFSHQPTWDDCQQLLQTLFTTEERERILLEARKNV
PGADGRPTQLQNEIDMGFPLTRPGWDYNTAEGRESLKIYRQALVAGLRGASRRPTNLAKVREVMQGPNEP
PSVFLERLMEAFRRFTPFDPTSEAQKASVALAFIGQSALDIRKKLQRLEGLQEAELRDLVREAEKVYYRR
ETEEEKEQRKEKEREE**reerrdrrqeknltk**ILAAVVEGKSSRERERDFRKIRSGPRQSGNLGNRTPLDK
DQCAYCKEKGHW**arncpkkgnkgpkv**LALEEDKD

FIGURE 1

```
PERVA      MHPTLSRRHLPIRGGKPKRLKIPLSFASIAWFLTLSITPQVNGKRLVDSPNSHKPLSLTW
POEVMSL    MHPTLNRRHLPIRGGKPKRLKIPLSFASIAWFLTLSITSQTNGMRIGDSLNSHKPLSLTW
TSUKUBA    MHPTLSRRHLPIRGGKPKRLKIPLSFASIAWFLTLSITSQTNGMRIGDSLNSHKPLSLTW
PERVB      MHPTLSWRHLPTRGGEPKRLRIPLSFASIAWFLTLTITPQASSKRLIDSSNPHRPLSLTW
POEV1      MHPTLSRRHLPTRGGEPKRLRIPLSFASIAWFLTLTITPQASSKRLIDSSNPHRPLSLTW
           *****. ***  :* :****:********:******:**.*... :: ** * *:*******


PERVA      LLTDSGTGININSTQGEAPLGTWWPELYVCLRSVIPGLNDQATPPDVLRAYGFYVCPGPP
POEVMSL    LITDSGTGININNTQGEAPLGTWWPDLYVCLRSVIPSL---TSPPDILHAHGFYVCPGPP
TSUKUBA    LITDSGTGININNTQGEAPLGTWWPDLYVCLRSVIPSL---TSPPDILHAHGFYVCPGPP
PERVB      LIIDPDTGVTVNSTRGVAPRGTWWPELHFCLRLINPAVK--STPPNLVRSYGFYCCPG-T
POEV1      LIIDPDTGVTVNSTRGVAPRGTWWPELHFCLRLINPAVK--STPPNLVRSYGFYCCPG-T
           *: *..**:.::.::* **  ****:*:.*** : *.:   ::.*::: :: *  ***. .


PERVA      NNEEYCGNPQDFFCKQWSCITSNDGNWKWPVSQQDRVSYSFVNNPTSYNQFNYGHGRWKD
POEVMSL    NNGKHCGNPRDFFCKQWNCVTSNDGYWKWPTSQQDRVSFSYVNTYTSSGQFNY-----LT
TSUKUBA    NNGKHCGNPRDFFCKQWNCVTSNDGYWKWPTSQQDRVSFSYVNTYTSSGQFNY-----LT
PERVB      EKEKYCGGSGESFCRRWSCVTSNDGDWKWPISLQDRVKFSFVNSG--PGKYKVMK-----
POEV1      EKEKYCGGSGESFCRRWSCVTSNDGDWKWPISLQDRVKFSFVNSG--PGKYKMMK-----
           :: :: *.. : * :: .*:*****  *** * ****.:*:**       :


PERVA      WQQRVQKDVRNKQISCHSLDLDYLKISFTE--KGKQENIQKWVNGISWGIVYYGGSGRKK
POEVMSL    W-------IRTGSPKCSPSDLDYLKISFTE--KGKQENILKWVNGMSWGMVYYGGSGKQP
TSUKUBA    W-------IRTGSPKCSPSDLDYLKISFTE--KGKQENILKWVNGMSWGMVYYGGSGKQP
PERVB      --------LYKDKSCSPSDLDYLKISFTE--KGKQENIQKWINGMSWGIVFYKYGGG-A
POEV1      --------LYKDKSCSPSDLDYLKISFTERKTGKYSKVDKWY---ELGNSFLLYGGG-A
                     . * . **********  . * .:: **     . *  :    .


PERVA      GSVLTIRLRIETQMEPPVAIGPNKGLAEQGPPIQEQRP-SPNPSD----------YNTT
POEVMSL    GSILTIRLKIN-QLEPPMAIGPNTVLTGQRPPTQ-----GPGPS----------SNIT
TSUKUBA    GSILTIRLKIN-QLEPPMAIGPNTVLTGQRPPTQ-----GPGPS----------SNIT
PERVB      GSTLTIRLRIETGTEPPVAVGPDKVLAEQGPPALEPPHNLPVPQLTSLRPDITQPPSNGT
POEV1      GSTLTIRLRIETGTEPPVAMGPDKVLAEQGPPALEPPHNLPVPQLTSLRPDITQPPSNST
           ** *****:*.   ***:*:**:. *: * .*          *.           : *


PERVA      SGSVPT-----EPNITIKTGAKLFSLIQGAFQALNSTTPEATSSCWLCLASGPPYYEGMA
POEVMSL    SGSDPT-----ESNSTTKMGAKLFSLIQGAFQALNSTTPEATSSCWLCLASGPPYYEGMA
TSUKUBA    SGSDPT-----ESSSTTKMGAKLFSLIQGAFQALNSTTPEATSSCWLCLALGPPYYEGMA
PERVB      TGLIPTNTPRNSPGVPVKTGQRLFSLIQGAFQAINSTDPDATSSCWLCLSSGPPYYEGMA
POEV1      TGLIPTNTPRNSPGVPVKTGQRLFSLIQGAFQAINSTDPDATSSCWLCLSSGPPYYEGMA
           :   *.      ... . * * :***********:*** *:**********: .****: **


PERVA      RGGKFNVTKEHRDQCTWGSQNKLTLTEVSGKGTCIGMVPPSHQHLCNHTEAFNRTSESQY
POEVMSL    RRGKFNVTKEHRDQCTWGSQNKLTLTEVSGKGTCIGKVPPSHQHLCNHTEAFNQTSESQY
TSUKUBA    RRGKFNVTKEHRDQCTWGSQNKLTLTEVSGKGTCIGKVPPSHQHLCNHTEAFNQTSESQY
PERVB      KEGKFNVTKEHRNQCTWGSRNKLTLTEVSGKGTCIGKAPPSHQHLCYSTVVYEQASENQY
POEV1      KERKFNVTKEHRNQCTWGSRNKLTLTEVSGKGTCIGKAPPSHQHLCYSTVVYEQASENQY
           :  ******:**:******:**********.***  ..******   * .::::**.**


PERVA      LVPGYDRWWACNTGLTPCVSTLVFNQTKDFCVMVQIVPRVYYYPEKAVLDEYDYRYNRPK
POEVMSL    LVPGYDRWWACNTGLTPCVSTLVFNQTKDFCIMVQIVPRVYYYPEKAILDEYDYRNHRQK
TSUKUBA    LVPGYDRWWACNTGLTPCVSTLVFNQTKDFCIMVQIVPRVYYYPEKAILDEYDYRNHRQK
PERVB      LVPGYNRWWACNTGLTPCVSTSVFNQSKDFCVMVQIVPRVYYHPEEVVLDEYDYRYNRPK
POEV1      LVPGYNRWWACNTGLTPCVSTSVFNQSKDFCVMVQIVPRVYYHPEEVVLDEYDYRYNRPK
           *****:**************** ****:*** :**********:*::.:******* :* *
```

```
PERVA       REPISLTLAVMLGLGVAAGVGTGTAALITGPQQLEKGLSNLHRIVTEDLQALEKSVSNLE
POEVMSL     REPISLTLAVMLGLGVAAGVGTGTAALVTGPQQLETGLSNLHRIVTEDLQALEKSVSNLE
TSUKUBA     REPISLTLAVMLGLGVAAGVGTGTAALVTGPQQLETGLSNLHRIVTEDLQALEKSVSNLE
PERVB       REPVSLTLAVMLGLGTAVGVGTGTAALITGPQQLEKGLGELHAAMTEDLRALEESVSNLE
POEV1       REPVSLTLAVMLGLGTAVGVGTGTAALITGPQQLEKGLGELHAAMTEDLRALKESVSNLE
            ::*:***********. .** ******:*******.**.:**  :* :*:**::*******

PERVA       ESLTSLSEVVLQNRRGLDLLFLKEGGLCVALKEECCFYVDHSGAIRDSMSKLRERLERRR
POEVMSL     ESLTSLSEVVLQNRRGLDLLFLKEGGLCVALKEECCFYVDHSGAIRDSMNKLRERLEKRR
TSUKUBA     ESLTSLSEVVLQNRRGLDLLFLKEGGLCVALKEECCFYVDHSGAIRDSMNKLRERLEKRR
PERVB       ESLTSLSEVVLQNRRGLDLLFLREGGLCAALKEECCFYVDHSGAIRDSMSKLRERLERRR
POEV1       ESLTSLSEVVLQNRRGLDLLFLREGGLCAALKEECCFYVDHSGAIRDSMNKLRKKLERRR
            :************::*******::  **.****:***************.**:::**:::*

PERVA       REREADQGWFEGWFNRSPWMTTLLSALTGPLVVLLLLLTVGPCLINRFVAFVRERVSAVQ
POEVMSL     REKETTQGWFEGWFNRSLWLATLLSALTGPLIVLLLLLTVGPCIINKLIAFIRERISAVQ
TSUKUBA     REKETTQGWFEGWFNRSPWLATLLSALTGPLIVLLLLLTVGPCIINKLIAFIRERISAVQ
PERVB       REREADQGWFEGWFNRSPWMTTLLSALTGPLVVLLLLLTVGPCLINRFVAFVRERVSAVQ
POEV1       REREADQGWFEGWFNRSPWMTTLLSALTGPLVVLLLLLTVGPCLINRFVAFVRERVSAVQ
            **:*: * **: *:*** *::****:*****:*******:***:**:::**:*:*:*****

PERVA       IMVLRQQYQGLLSQGETDL
POEVMSL     IMVLRQQYQSPSSR-EAGR
TSUKUBA     IMVLRQQYQSPSSR-EAGR
PERVB       IMVLRQQYQGLLSQGETDL
POEV1       IMVLRQQYQGLLSQGETDL
            *********.  .: *:.
```

FIGURE 2

```
PERVA     NNEEYCGNPQDFFCKQWSCITSNDGNWKWPVSQQDRVSYSFVNNPTSYNQFNYghgrwkd
POEVMSL   NNGKHCGNPRDFFCKQWNCVTSNDGYWKWPTSQQDRVSFSYVNTYTSSGQFNY-----LT
TSUKUBA   NNGKHCGNPRDFFCKQWNCVTSNDGYWKWPTSQQDRVSFSYVNTYTSSGQFNY-----LT
PERVB     EKEKYCGGSGESFCRRWSCVTSNDGDWKWPISLQDRVKFSFVNSG--PGKYKVMK-----
POEV1     EKEKYCGGSGESFCRRWSCVTSNDGDWKWPISLQDRVKFSFVNSG--PGKYKMMK-----

PERVA     wqqrvqkdvrnkqisCHSLDLDYLKISFTE--KGKQENIQKWVNGISWGIVYYGGSGRKK
POEVMSL   W-------IRTGSPKCSPSDLDYLKISFTE--KGKQENILKWVNGMSWGMVYYGGSGKQP
TSUKUBA   W-------IRTGSPKCSPSDLDYLKISFTE--KGKQENILKWVNGMSWGMVYYGGSGKQP
PERVB     ---------LYKDKSCSPSDLDYLKISFTE--kgkqeniqkwingmsWGIVFYKYGGG-A
POEV1     ---------LYKDKSCSPSDLDYLKISFTErktgkyskvdkwy---elgnsFLLYGGG-A

PERVA     GSVLTIRLRIETQMEPPVAIGPNKGLAEQGPPiqeqrp-spnpsd----------yntt
POEVMSL   GSILTIRLKIN-QLEPPMAIGPNTVLTGQRPPTQ-----GPGPS-----------SNIT
TSUKUBA   GSILTIRLKIN-QLEPPMAIGPntvltgqrpptq-----GPGPS-----------SNIT
PERVB     GSTLTIRLRIETGTEPPVAVGPDKVLAEQGPPALEPPHNLPVPQLTSLRPDITQPPSNGT
POEV1     GSTLTIRLRIETGTEPPVAMGPDKVLAEQGPPALEPPHNLPVPQLtslrpditqppsnst

PERVA     SGSVPT-----EPNITIKTGAKLFSLIQGAFQALNSTTPEATSSCWLCLASGPPYYEGMA
POEVMSL   SGSDPT-----ESNSTTKMGAKLFSLIQGAFQALNSTTPEATSSCWLCLASGPPYYEGMA
TSUKUBA   SGSDPT-----ESSSTTKMGAKLFSLIQGAFQALNSTTPEATSSCWLCLALGPPYYEGMA
PERVB     TGLIPTNTPRNSPGVPVKTGQRLFSLIQGAFQAINSTDPDATSSCWLCLSSGPPYYEGMA
POEV1     TGLIPTNTPRNSPGVPVKTGQRLFSLIQGAFQAINSTDPDATSSCWLCLSSGPPYYEGMA
```

FIGURE 3

ATTORNEY DOCKET NO. **9013.43**

DECLARATION, POWER OF ATTORNEY AND PETITION

As the below named inventor, I hereby declare:

my residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled _____ RETROVIRUS ASSAY _____, the specification of which

[ ] is attached hereto.

[X] was filed on **Feb. 15, 2002**,
as Application Serial No. **10/069,082**
and was amended on _____ (if applicable).

[X] was described and claimed in PCT International Application No. PCT/GB00/03159
and as amended under PCT Article 19 on _____ (if any).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent & Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

PRIORITY CLAIM UNDER 35 USC § 119(a)-(d)

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign applications(s) for patent or inventor's certificate, or §365(a) of any PCT International Applications designating at least one country other than the U.S. listed below and have also identified below any foreign application for patent or inventor's certificate or of any PCT International Applications designating at least one country other than the U.S. having a filing date before that of the application on which priority is claimed:

[ ] no such applications have been filed

[X] application(s) listed below:

PRIOR FOREIGN APPLICATIONS(S)
Filed Within Twelve Months (Six Months For Design) Of This Application

			PRIORITY CLAIMED YES	NO
9919604.0	United Kingdom	18 August 1999	[X]	[ ]
(Number)	(Country)	(Day/month/year filed)		
_____			[ ]	[ ]
(Number)	(Country)	(Day/month/year filed)		
_____			[ ]	[ ]
(Number)	(Country)	(Day/month/year filed)		

CLAIM FOR BENEFIT OF PROVISIONAL APPLICATION UNDER 35 USC §119(e)

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States Provisional application listed below:

PROVISIONAL APPLICATION NO.	FILING DATE
_____	_____
_____	_____

CLAIM FOR BENEFIT OF EARLIER APPLICATIONS UNDER 35 USC §120

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s), or §365(c) of any PCT International Application(s) designating the U.S. listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the U.S. Patent & Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(App. Serial No.)	(Filing date)	(Status) (patented, pending, abandoned)
(App. Serial No.)	(Filing date)	(Status) (patented, pending, abandoned)

PRIOR FOREIGN APPLICATIONS
(Filed More Than Twelve Months (Six Months for Design) Prior To This Application

(Number)	(Country)	(Day/month/year filed)
(Number)	(Country)	(Day/month/year filed)
(Number)	(Country)	(Day/month/year filed)

POWER OF ATTORNEY

And I hereby appoint the practitioners associated with the Customer Number provided below as my attorneys, to prosecute this application and to transact all business in the United States Patent and Trademark Office connected therewith, and direct that all correspondence be addressed to that Customer Number:

Customer Number 20792

Send correspondence to:	Kenneth D Sibley Myers Bigel Sibley & Sajovec Post Office Box 37428 Raleigh, NC 27627
Direct telephone calls to:	Kenneth D Sibley (919) 854 1400
Facsimile:	(919) 854 1401

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Inventor's signature

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Inventor's signature

Date 4th October 2002 Citizenship British

Residence Glasgow, United Kingdom GBX

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Full name of third, joint inventor, if any Kenneth SMITH

Inventor's signature

Date 4th October 2002 Citizenship British

Residence Glasgow, United Kingdom GBX

Post Office Address 5 Capelrig Lane, Newton Mearns, Glasgow, G77 6XZ, United Kingdom

SEQUENCE LISTING

<110> Quip Technology Limited

<120> Retrovirus Assay

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<140> US 10/069,082
<141> 2000-08-16

<150> GB 9919604.0
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Gly Trp Pro Ser Glu Gly Thr Phe Asn Ser Glu Ile Ile Leu Ala Val
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Lys Ala Ile Ile Phe Gln Thr Gly Pro Gly Ser His Pro Asp Gln Glu
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Pro Tyr Ile Leu Thr Trp Gln Asp Leu Ala Glu Asp Pro Pro Pro Trp
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Val Lys Pro Trp Leu Asn Lys Pro Arg Lys Pro Gly Pro Arg Ile Leu
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Ser Ser Tyr Leu Pro Arg Asp Arg Gly Ala Ala Asp Leu Ala Gly Thr
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Pro Thr Cys Ser Pro Thr Pro Leu Ser Ser Thr Gly Cys Cys Glu Gly
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180 185 190

Ile Leu Pro Leu Arg Thr Tyr Gly Pro Pro Met Pro Gly Gly Gln Leu
195 200 205

Gln Pro Leu Gln Tyr Trp Pro Phe Ser Ser Ala Asp Leu Tyr Asn Trp
210 215 220

Lys Thr Asn His Pro Pro Phe Ser Glu Asp Pro Gln Arg Leu Thr Gly
225 230 235 240

Leu Val Glu Ser Leu Met Phe Ser His Gln Pro Thr Trp Asp Asp Cys
245 250 255

Gln Gln Leu Leu Gln Thr Leu Phe Thr Thr Glu Glu Arg Glu Arg Ile
260 265 270

Leu Leu Glu Ala Arg Lys Asn Val Pro Gly Ala Asp Gly Arg Pro Thr
275 280 285

Gln Leu Gln Asn Glu Ile Asp Met Gly Phe Pro Leu Thr Arg Pro Gly
290 295 300

Trp Asp Tyr Asn Thr Ala Glu Gly Arg Glu Ser Leu Lys Ile Tyr Arg
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Gln Ala Leu Val Ala Gly Leu Arg Gly Ala Ser Arg Arg Pro Thr Asn
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Leu Ala Lys Val Arg Glu Val Met Gln Gly Pro Asn Glu Pro Pro Ser
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Val Phe Leu Glu Arg Leu Met Glu Ala Phe Arg Arg Phe Thr Pro Phe
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Asp Pro Thr Ser Glu Ala Gln Lys Ala Ser Val Ala Leu Ala Phe Ile
370 375 380

Gly Gln Ser Ala Leu Asp Ile Arg Lys Lys Leu Gln Arg Leu Glu Gly
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Leu Gln Glu Ala Glu Leu Arg Asp Leu Val Arg Glu Ala Glu Lys Val
405 410 415

Tyr Tyr Arg Arg Glu Thr Glu Glu Glu Lys Glu Gln Arg Lys Glu Lys
420 425 430

Glu Arg Glu Glu Arg Glu Glu Arg Arg Asp Arg Arg Gln Glu Lys Asn
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Leu Thr Lys Ile Leu Ala Ala Val Val Glu Gly Lys Ser Ser Arg Glu
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Arg Glu Arg Asp Phe Arg Lys Ile Arg Ser Gly Pro Arg Gln Ser Gly
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Asn Leu Gly Asn Arg Thr Pro Leu Asp Lys Asp Gln Cys Ala Tyr Cys
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35 40 45

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Ser Pro Asn Ser His Lys Pro Leu Ser Leu Thr Trp Leu Leu Thr Asp
     50                  55                  60

Ser Gly Thr Gly Ile Asn Ile Asn Ser Thr Gln Gly Glu Ala Pro Leu
 65                  70                  75                  80

Gly Thr Trp Trp Pro Glu Leu Tyr Val Cys Leu Arg Ser Val Ile Pro
                 85                  90                  95

Gly Leu Asn Asp Gln Ala Thr Pro Pro Asp Val Leu Arg Ala Tyr Gly
            100                 105                 110

Phe Tyr Val Cys Pro Gly Pro Pro Asn Asn Glu Glu Tyr Cys Gly Asn
        115                 120                 125

Pro Gln Asp Phe Phe Cys Lys Gln Trp Ser Cys Ile Thr Ser Asn Asp
    130                 135                 140

Gly Asn Trp Lys Trp Pro Val Ser Gln Gln Asp Arg Val Ser Tyr Ser
145                 150                 155                 160

Phe Val Asn Asn Pro Thr Ser Tyr Asn Gln Phe Asn Tyr Gly His Gly
                165                 170                 175

Arg Trp Lys Asp Trp Gln Gln Arg Val Gln Lys Asp Val Arg Asn Lys
            180                 185                 190

Gln Ile Ser Cys His Ser Leu Asp Leu Asp Tyr Leu Lys Ile Ser Phe
        195                 200                 205

Thr Glu Lys Gly Lys Gln Glu Asn Ile Gln Lys Trp Val Asn Gly Ile
    210                 215                 220

Ser Trp Gly Ile Val Tyr Tyr Gly Gly Ser Gly Arg Lys Lys Gly Ser
225                 230                 235                 240

Val Leu Thr Ile Arg Leu Arg Ile Glu Thr Gln Met Glu Pro Pro Val
                245                 250                 255

Ala Ile Gly Pro Asn Lys Gly Leu Ala Glu Gln Gly Pro Pro Ile Gln
            260                 265                 270

Glu Gln Arg Pro Ser Pro Asn Pro Ser Asp Tyr Asn Thr Thr Ser Gly
        275                 280                 285

Ser Val Pro Thr Glu Pro Asn Ile Thr Ile Lys Thr Gly Ala Lys Leu
    290                 295                 300

Phe Ser Leu Ile Gln Gly Ala Phe Gln Ala Leu Asn Ser Thr Thr Pro
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Glu Ala Thr Ser Ser Cys Trp Leu Cys Leu Ala Ser Gly Pro Pro Tyr
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His Leu Cys Asn His Thr Glu Ala Phe Asn Arg Thr Ser Glu Ser Gln
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Ile Ser Leu Thr Leu Ala Val Met Leu Gly Leu Gly Val Ala Ala Gly
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Val Gly Thr Gly Thr Ala Ala Leu Ile Thr Gly Pro Gln Gln Leu Glu
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Lys Gly Leu Ser Asn Leu His Arg Ile Val Thr Glu Asp Leu Gln Ala
500 505 510

Leu Glu Lys Ser Val Ser Asn Leu Glu Glu Ser Leu Thr Ser Leu Ser
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Glu Val Val Leu Gln Asn Arg Arg Gly Leu Asp Leu Leu Phe Leu Lys
530 535 540

Glu Gly Gly Leu Cys Val Ala Leu Lys Glu Glu Cys Cys Phe Tyr Val
545 550 555 560

Asp His Ser Gly Ala Ile Arg Asp Ser Met Ser Lys Leu Arg Glu Arg
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Leu Glu Arg Arg Arg Arg Glu Arg Glu Ala Asp Gln Gly Trp Phe Glu
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Gly Trp Phe Asn Arg Ser Pro Trp Met Thr Thr Leu Leu Ser Ala Leu
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Leu Thr Leu Ser Ile Thr Ser Gln Thr Asn Gly Met Arg Ile Gly Asp
35 40 45

Ser Leu Asn Ser His Lys Pro Leu Ser Leu Thr Trp Leu Ile Thr Asp
50 55 60

Ser Gly Thr Gly Ile Asn Ile Asn Asn Thr Gln Gly Glu Ala Pro Leu
65 70 75 80

Gly Thr Trp Trp Pro Asp Leu Tyr Val Cys Leu Arg Ser Val Ile Pro
85 90 95

Ser Leu Thr Ser Pro Pro Asp Ile Leu His Ala His Gly Phe Tyr Val
100 105 110

Cys Pro Gly Pro Pro Asn Asn Gly Lys His Cys Gly Asn Pro Arg Asp
115 120 125

Phe Phe Cys Lys Gln Trp Asn Cys Val Thr Ser Asn Asp Gly Tyr Trp
130 135 140

Lys Trp Pro Thr Ser Gln Gln Asp Arg Val Ser Phe Ser Tyr Val Asn
145 150 155 160

Thr Tyr Thr Ser Ser Gly Gln Phe Asn Tyr Leu Thr Trp Ile Arg Thr
165 170 175

```
Gly Ser Pro Lys Cys Ser Pro Ser Asp Leu Asp Tyr Leu Lys Ile Ser
            180                 185                 190

Phe Thr Glu Lys Gly Lys Gln Glu Asn Ile Leu Lys Trp Val Asn Gly
        195                 200                 205

Met Ser Trp Gly Met Val Tyr Tyr Gly Gly Ser Gly Lys Gln Pro Gly
    210                 215                 220

Ser Ile Leu Thr Ile Arg Leu Lys Ile Asn Gln Leu Glu Pro Pro Met
225                 230                 235                 240

Ala Ile Gly Pro Asn Thr Val Leu Thr Gly Gln Arg Pro Pro Thr Gln
                245                 250                 255

Gly Pro Gly Pro Ser Ser Asn Ile Thr Ser Gly Ser Asp Pro Thr Glu
            260                 265                 270

Ser Asn Ser Thr Thr Lys Met Gly Ala Lys Leu Phe Ser Leu Ile Gln
        275                 280                 285

Gly Ala Phe Gln Ala Leu Asn Ser Thr Thr Pro Glu Ala Thr Ser Ser
    290                 295                 300

Cys Trp Leu Cys Leu Ala Ser Gly Pro Pro Tyr Tyr Glu Gly Met Ala
305                 310                 315                 320

Arg Arg Gly Lys Phe Asn Val Thr Lys Glu His Arg Asp Gln Cys Thr
                325                 330                 335

Trp Gly Ser Gln Asn Lys Leu Thr Leu Thr Glu Val Ser Gly Lys Gly
            340                 345                 350

Thr Cys Ile Gly Lys Val Pro Pro Ser His Gln His Leu Cys Asn His
        355                 360                 365

Thr Glu Ala Phe Asn Gln Thr Ser Glu Ser Gln Tyr Leu Val Pro Gly
    370                 375                 380

Tyr Asp Arg Trp Trp Ala Cys Asn Thr Gly Leu Thr Pro Cys Val Ser
385                 390                 395                 400

Thr Leu Val Phe Asn Gln Thr Lys Asp Phe Cys Ile Met Val Gln Ile
                405                 410                 415

Val Pro Arg Val Tyr Tyr Tyr Pro Glu Lys Ala Ile Leu Asp Glu Tyr
            420                 425                 430

Asp Tyr Arg Asn His Arg Gln Lys Arg Glu Pro Ile Ser Leu Thr Leu
        435                 440                 445
```

```
Ala Val Met Leu Gly Leu Gly Val Ala Ala Gly Val Gly Thr Gly Thr
    450                 455                 460

Ala Ala Leu Val Thr Gly Pro Gln Gln Leu Glu Thr Gly Leu Ser Asn
465                 470                 475                 480

Leu His Arg Ile Val Thr Glu Asp Leu Gln Ala Leu Glu Lys Ser Val
                485                 490                 495

Ser Asn Leu Glu Glu Ser Leu Thr Ser Leu Ser Glu Val Val Leu Gln
            500                 505                 510

Asn Arg Arg Gly Leu Asp Leu Leu Phe Leu Lys Glu Gly Gly Leu Cys
        515                 520                 525

Val Ala Leu Lys Glu Glu Cys Cys Phe Tyr Val Asp His Ser Gly Ala
    530                 535                 540

Ile Arg Asp Ser Met Asn Lys Leu Arg Glu Arg Leu Glu Lys Arg Arg
545                 550                 555                 560

Arg Glu Lys Glu Thr Thr Gln Gly Trp Phe Glu Gly Trp Phe Asn Arg
                565                 570                 575

Ser Leu Trp Leu Ala Thr Leu Leu Ser Ala Leu Thr Gly Pro Leu Ile
            580                 585                 590

Val Leu Leu Leu Leu Leu Thr Val Gly Pro Cys Ile Ile Asn Lys Leu
        595                 600                 605

Ile Ala Phe Ile Arg Glu Arg Ile Ser Ala Val Gln Ile Met Val Leu
    610                 615                 620

Arg Gln Gln Tyr Gln Ser Pro Ser Ser Arg Glu Ala Gly Arg
625                 630                 635
```

<210> 4

<211> 638

<212> PRT

<213> porcine endogenous retrovirus Type Tsukuba

<400> 4

```
Met His Pro Thr Leu Ser Arg Arg His Leu Pro Ile Arg Gly Gly Lys
1               5                   10                  15

Pro Lys Arg Leu Lys Ile Pro Leu Ser Phe Ala Ser Ile Ala Trp Phe
            20                  25                  30

Leu Thr Leu Ser Ile Thr Ser Gln Thr Asn Gly Met Arg Ile Gly Asp
        35                  40                  45
```

```
Ser Leu Asn Ser His Lys Pro Leu Ser Leu Thr Trp Leu Ile Thr Asp
 50                  55                  60

Ser Gly Thr Gly Ile Asn Ile Asn Asn Thr Gln Gly Glu Ala Pro Leu
65                  70                  75                  80

Gly Thr Trp Trp Pro Asp Leu Tyr Val Cys Leu Arg Ser Val Ile Pro
                85                  90                  95

Ser Leu Thr Ser Pro Pro Asp Ile Leu His Ala His Gly Phe Tyr Val
            100                 105                 110

Cys Pro Gly Pro Pro Asn Asn Gly Lys His Cys Gly Asn Pro Arg Asp
        115                 120                 125

Phe Phe Cys Lys Gln Trp Asn Cys Val Thr Ser Asn Asp Gly Tyr Trp
    130                 135                 140

Lys Trp Pro Thr Ser Gln Gln Asp Arg Val Ser Phe Ser Tyr Val Asn
145                 150                 155                 160

Thr Tyr Thr Ser Ser Gly Gln Phe Asn Tyr Leu Thr Trp Ile Arg Thr
                165                 170                 175

Gly Ser Pro Lys Cys Ser Pro Ser Asp Leu Asp Tyr Leu Lys Ile Ser
            180                 185                 190

Phe Thr Glu Lys Gly Lys Gln Glu Asn Ile Leu Lys Trp Val Asn Gly
        195                 200                 205

Met Ser Trp Gly Met Val Tyr Tyr Gly Gly Ser Gly Lys Gln Pro Gly
    210                 215                 220

Ser Ile Leu Thr Ile Arg Leu Lys Ile Asn Gln Leu Glu Pro Pro Met
225                 230                 235                 240

Ala Ile Gly Pro Asn Thr Val Leu Thr Gly Gln Arg Pro Pro Thr Gln
                245                 250                 255

Gly Pro Gly Pro Ser Ser Asn Ile Thr Ser Gly Ser Asp Pro Thr Glu
            260                 265                 270

Ser Ser Ser Thr Thr Lys Met Gly Ala Lys Leu Phe Ser Leu Ile Gln
        275                 280                 285

Gly Ala Phe Gln Ala Leu Asn Ser Thr Thr Pro Glu Ala Thr Ser Ser
    290                 295                 300

Cys Trp Leu Cys Leu Ala Leu Gly Pro Pro Tyr Tyr Glu Gly Met Ala
305                 310                 315                 320
```

Arg Arg Gly Lys Phe Asn Val Thr Lys Glu His Arg Asp Gln Cys Thr
325 330 335

Trp Gly Ser Gln Asn Lys Leu Thr Leu Thr Glu Val Ser Gly Lys Gly
340 345 350

Thr Cys Ile Gly Lys Val Pro Pro Ser His Gln His Leu Cys Asn His
355 360 365

Thr Glu Ala Phe Asn Gln Thr Ser Glu Ser Gln Tyr Leu Val Pro Gly
370 375 380

Tyr Asp Arg Trp Trp Ala Cys Asn Thr Gly Leu Thr Pro Cys Val Ser
385 390 395 400

Thr Leu Val Phe Asn Gln Thr Lys Asp Phe Cys Ile Met Val Gln Ile
405 410 415

Val Pro Arg Val Tyr Tyr Tyr Pro Glu Lys Ala Ile Leu Asp Glu Tyr
420 425 430

Asp Tyr Arg Asn His Arg Gln Lys Arg Glu Pro Ile Ser Leu Thr Leu
435 440 445

Ala Val Met Leu Gly Leu Gly Val Ala Ala Gly Val Gly Thr Gly Thr
450 455 460

Ala Ala Leu Val Thr Gly Pro Gln Gln Leu Glu Thr Gly Leu Ser Asn
465 470 475 480

Leu His Arg Ile Val Thr Glu Asp Leu Gln Ala Leu Glu Lys Ser Val
485 490 495

Ser Asn Leu Glu Glu Ser Leu Thr Ser Leu Ser Glu Val Val Leu Gln
500 505 510

Asn Arg Arg Gly Leu Asp Leu Leu Phe Leu Lys Glu Gly Gly Leu Cys
515 520 525

Val Ala Leu Lys Glu Glu Cys Cys Phe Tyr Val Asp His Ser Gly Ala
530 535 540

Ile Arg Asp Ser Met Asn Lys Leu Arg Glu Arg Leu Glu Lys Arg Arg
545 550 555 560

Arg Glu Lys Glu Thr Thr Gln Gly Trp Phe Glu Gly Trp Phe Asn Arg
565 570 575

Ser Pro Trp Leu Ala Thr Leu Leu Ser Ala Leu Thr Gly Pro Leu Ile
580 585 590

Val Leu Leu Leu Leu Leu Thr Val Gly Pro Cys Ile Ile Asn Lys Leu
595 600 605

Ile Ala Phe Ile Arg Glu Arg Ile Ser Ala Val Gln Ile Met Val Leu
610 615 620

Arg Gln Gln Tyr Gln Ser Pro Ser Ser Arg Glu Ala Gly Arg
625 630 635

<210> 5

<211> 657

<212> PRT

<213> porcine endogenous retrovirus Type PERV B

<400> 5

Met His Pro Thr Leu Ser Trp Arg His Leu Pro Thr Arg Gly Gly Glu
1 5 10 15

Pro Lys Arg Leu Arg Ile Pro Leu Ser Phe Ala Ser Ile Ala Trp Phe
20 25 30

Leu Thr Leu Thr Ile Thr Pro Gln Ala Ser Ser Lys Arg Leu Ile Asp
35 40 45

Ser Ser Asn Pro His Arg Pro Leu Ser Leu Thr Trp Leu Ile Ile Asp
50 55 60

Pro Asp Thr Gly Val Thr Val Asn Ser Thr Arg Gly Val Ala Pro Arg
65 70 75 80

Gly Thr Trp Trp Pro Glu Leu His Phe Cys Leu Arg Leu Ile Asn Pro
85 90 95

Ala Val Lys Ser Thr Pro Pro Asn Leu Val Arg Ser Tyr Gly Phe Tyr
100 105 110

Cys Cys Pro Gly Thr Glu Lys Glu Lys Tyr Cys Gly Gly Ser Gly Glu
115 120 125

Ser Phe Cys Arg Arg Trp Ser Cys Val Thr Ser Asn Asp Gly Asp Trp
130 135 140

Lys Trp Pro Ile Ser Leu Gln Asp Arg Val Lys Phe Ser Phe Val Asn
145 150 155 160

Ser Gly Pro Gly Lys Tyr Lys Val Met Lys Leu Tyr Lys Asp Lys Ser
165 170 175

Cys Ser Pro Ser Asp Leu Asp Tyr Leu Lys Ile Ser Phe Thr Glu Lys
180 185 190

```
Gly Lys Gln Glu Asn Ile Gln Lys Trp Ile Asn Gly Met Ser Trp Gly
        195                 200                 205

Ile Val Phe Tyr Lys Tyr Gly Gly Gly Ala Gly Ser Thr Leu Thr Ile
    210                 215                 220

Arg Leu Arg Ile Glu Thr Gly Thr Glu Pro Pro Val Ala Val Gly Pro
225                 230                 235                 240

Asp Lys Val Leu Ala Glu Gln Gly Pro Pro Ala Leu Glu Pro Pro His
                245                 250                 255

Asn Leu Pro Val Pro Gln Leu Thr Ser Leu Arg Pro Asp Ile Thr Gln
            260                 265                 270

Pro Pro Ser Asn Gly Thr Thr Gly Leu Ile Pro Thr Asn Thr Pro Arg
        275                 280                 285

Asn Ser Pro Gly Val Pro Val Lys Thr Gly Gln Arg Leu Phe Ser Leu
    290                 295                 300

Ile Gln Gly Ala Phe Gln Ala Ile Asn Ser Thr Asp Pro Asp Ala Thr
305                 310                 315                 320

Ser Ser Cys Trp Leu Cys Leu Ser Ser Gly Pro Pro Tyr Tyr Glu Gly
                325                 330                 335

Met Ala Lys Glu Gly Lys Phe Asn Val Thr Lys Glu His Arg Asn Gln
            340                 345                 350

Cys Thr Trp Gly Ser Arg Asn Lys Leu Thr Leu Thr Glu Val Ser Gly
        355                 360                 365

Lys Gly Thr Cys Ile Gly Lys Ala Pro Pro Ser His Gln His Leu Cys
    370                 375                 380

Tyr Ser Thr Val Val Tyr Glu Gln Ala Ser Glu Asn Gln Tyr Leu Val
385                 390                 395                 400

Pro Gly Tyr Asn Arg Trp Trp Ala Cys Asn Thr Gly Leu Thr Pro Cys
                405                 410                 415

Val Ser Thr Ser Val Phe Asn Gln Ser Lys Asp Phe Cys Val Met Val
            420                 425                 430

Gln Ile Val Pro Arg Val Tyr Tyr His Pro Glu Glu Val Val Leu Asp
        435                 440                 445

Glu Tyr Asp Tyr Arg Tyr Asn Arg Pro Lys Arg Glu Pro Val Ser Leu
    450                 455                 460

Thr Leu Ala Val Met Leu Gly Leu Gly Thr Ala Val Gly Val Gly Thr
465                 470                 475                 480
```

Gly Thr Ala Ala Leu Ile Thr Gly Pro Gln Gln Leu Glu Lys Gly Leu
485 490 495

Gly Glu Leu His Ala Ala Met Thr Glu Asp Leu Arg Ala Leu Glu Glu
500 505 510

Ser Val Ser Asn Leu Glu Glu Ser Leu Thr Ser Leu Ser Glu Val Val
515 520 525

Leu Gln Asn Arg Arg Gly Leu Asp Leu Leu Phe Leu Arg Glu Gly Gly
530 535 540

Leu Cys Ala Ala Leu Lys Glu Glu Cys Cys Phe Tyr Val Asp His Ser
545 550 555 560

Gly Ala Ile Arg Asp Ser Met Ser Lys Leu Arg Glu Arg Leu Glu Arg
565 570 575

Arg Arg Arg Glu Arg Glu Ala Asp Gln Gly Trp Phe Glu Gly Trp Phe
580 585 590

Asn Arg Ser Pro Trp Met Thr Thr Leu Leu Ser Ala Leu Thr Gly Pro
595 600 605

Leu Val Val Leu Leu Leu Leu Leu Thr Val Gly Pro Cys Leu Ile Asn
610 615 620

Arg Phe Val Ala Phe Val Arg Glu Arg Val Ser Ala Val Gln Ile Met
625 630 635 640

Val Leu Arg Gln Gln Tyr Gln Gly Leu Leu Ser Gln Gly Glu Thr Asp
645 650 655

Leu

<210> 6

<211> 656

<212> PRT

<213> porcine endogenous retrovirus Type PoEV1

<400> 6

Met His Pro Thr Leu Ser Arg Arg His Leu Pro Thr Arg Gly Gly Glu
1 5 10 15

Pro Lys Arg Leu Arg Ile Pro Leu Ser Phe Ala Ser Ile Ala Trp Phe
20 25 30

```
Leu Thr Leu Thr Ile Thr Pro Gln Ala Ser Ser Lys Arg Leu Ile Asp
        35                  40                  45

Ser Ser Asn Pro His Arg Pro Leu Ser Leu Thr Trp Leu Ile Ile Asp
    50                  55                  60

Pro Asp Thr Gly Val Thr Val Asn Ser Thr Arg Gly Val Ala Pro Arg
65                  70                  75                  80

Gly Thr Trp Trp Pro Glu Leu His Phe Cys Leu Arg Leu Ile Asn Pro
                85                  90                  95

Ala Val Lys Ser Thr Pro Pro Asn Leu Val Arg Ser Tyr Gly Phe Tyr
            100                 105                 110

Cys Cys Pro Gly Thr Glu Lys Glu Lys Tyr Cys Gly Gly Ser Gly Glu
        115                 120                 125

Ser Phe Cys Arg Arg Trp Ser Cys Val Thr Ser Asn Asp Gly Asp Trp
    130                 135                 140

Lys Trp Pro Ile Ser Leu Gln Asp Arg Val Lys Phe Ser Phe Val Asn
145                 150                 155                 160

Ser Gly Pro Gly Lys Tyr Lys Met Met Lys Leu Tyr Lys Asp Lys Ser
                165                 170                 175

Cys Ser Pro Ser Asp Leu Asp Tyr Leu Lys Ile Ser Phe Thr Glu Arg
            180                 185                 190

Lys Thr Gly Lys Tyr Ser Lys Val Asp Lys Trp Tyr Glu Leu Gly Asn
        195                 200                 205

Ser Phe Leu Leu Tyr Gly Gly Gly Ala Gly Ser Thr Leu Thr Ile Arg
    210                 215                 220

Leu Arg Ile Glu Thr Gly Thr Glu Pro Pro Val Ala Met Gly Pro Asp
225                 230                 235                 240

Lys Val Leu Ala Glu Gln Gly Pro Pro Ala Leu Glu Pro Pro His Asn
                245                 250                 255

Leu Pro Val Pro Gln Leu Thr Ser Leu Arg Pro Asp Ile Thr Gln Pro
            260                 265                 270

Pro Ser Asn Ser Thr Thr Gly Leu Ile Pro Thr Asn Thr Pro Arg Asn
        275                 280                 285

Ser Pro Gly Val Pro Val Lys Thr Gly Gln Arg Leu Phe Ser Leu Ile
    290                 295                 300

Gln Gly Ala Phe Gln Ala Ile Asn Ser Thr Asp Pro Asp Ala Thr Ser
305                 310                 315                 320
```

Ser Cys Trp Leu Cys Leu Ser Ser Gly Pro Pro Tyr Tyr Glu Gly Met
325 330 335

Ala Lys Glu Arg Lys Phe Asn Val Thr Lys Glu His Arg Asn Gln Cys
340 345 350

Thr Trp Gly Ser Arg Asn Lys Leu Thr Leu Thr Glu Val Ser Gly Lys
355 360 365

Gly Thr Cys Ile Gly Lys Ala Pro Pro Ser His Gln His Leu Cys Tyr
370 375 380

Ser Thr Val Val Tyr Glu Gln Ala Ser Glu Asn Gln Tyr Leu Val Pro
385 390 395 400

Gly Tyr Asn Arg Trp Trp Ala Cys Asn Thr Gly Leu Thr Pro Cys Val
405 410 415

Ser Thr Ser Val Phe Asn Gln Ser Lys Asp Phe Cys Val Met Val Gln
420 425 430

Ile Val Pro Arg Val Tyr Tyr His Pro Glu Glu Val Val Leu Asp Glu
435 440 445

Tyr Asp Tyr Arg Tyr Asn Arg Pro Lys Arg Glu Pro Val Ser Leu Thr
450 455 460

Leu Ala Val Met Leu Gly Leu Gly Thr Ala Val Gly Val Gly Thr Gly
465 470 475 480

Thr Ala Ala Leu Ile Thr Gly Pro Gln Gln Leu Glu Lys Gly Leu Gly
485 490 495

Glu Leu His Ala Ala Met Thr Glu Asp Leu Arg Ala Leu Lys Glu Ser
500 505 510

Val Ser Asn Leu Glu Glu Ser Leu Thr Ser Leu Ser Glu Val Val Leu
515 520 525

Gln Asn Arg Arg Gly Leu Asp Leu Leu Phe Leu Arg Glu Gly Gly Leu
530 535 540

Cys Ala Ala Leu Lys Glu Glu Cys Cys Phe Tyr Val Asp His Ser Gly
545 550 555 560

Ala Ile Arg Asp Ser Met Asn Lys Leu Arg Lys Lys Leu Glu Arg Arg
565 570 575

Arg Arg Glu Arg Glu Ala Asp Gln Gly Trp Phe Glu Gly Trp Phe Asn
580 585 590

Arg Ser Pro Trp Met Thr Thr Leu Leu Ser Ala Leu Thr Gly Pro Leu
595 600 605

Val Val Leu Leu Leu Leu Leu Thr Val Gly Pro Cys Leu Ile Asn Arg
610 615 620

Phe Val Ala Phe Val Arg Glu Arg Val Ser Ala Val Gln Ile Met Val
625 630 635 640

Leu Arg Gln Gln Tyr Gln Gly Leu Leu Ser Gln Gly Glu Thr Asp Leu
645 650 655

<210> 7

<211> 221

<212> PRT

<213> porcine endogenous retrovirus Type PERV A

<400> 7

Asn Asn Glu Glu Tyr Cys Gly Asn Pro Gln Asp Phe Phe Cys Lys Gln
1 5 10 15

Trp Ser Cys Ile Thr Ser Asn Asp Gly Asn Trp Lys Trp Pro Val Ser
20 25 30

Gln Gln Asp Arg Val Ser Tyr Ser Phe Val Asn Asn Pro Thr Ser Tyr
35 40 45

Asn Gln Phe Asn Tyr Gly His Gly Arg Trp Lys Asp Trp Gln Gln Arg
50 55 60

Val Gln Lys Asp Val Arg Asn Lys Gln Ile Ser Cys His Ser Leu Asp
65 70 75 80

Leu Asp Tyr Leu Lys Ile Ser Phe Thr Glu Lys Gly Lys Gln Glu Asn
85 90 95

Ile Gln Lys Trp Val Asn Gly Ile Ser Trp Gly Ile Val Tyr Tyr Gly
100 105 110

Gly Ser Gly Arg Lys Lys Gly Ser Val Leu Thr Ile Arg Leu Arg Ile
115 120 125

Glu Thr Gln Met Glu Pro Pro Val Ala Ile Gly Pro Asn Lys Gly Leu
130 135 140

Ala Glu Gln Gly Pro Pro Ile Gln Glu Gln Arg Pro Ser Pro Asn Pro
145 150 155 160

Ser Asp Tyr Asn Thr Thr Ser Gly Ser Val Pro Thr Glu Pro Asn Ile
165 170 175

Thr Ile Lys Thr Gly Ala Lys Leu Phe Ser Leu Ile Gln Gly Ala Phe
180 185 190

Gln Ala Leu Asn Ser Thr Thr Pro Glu Ala Thr Ser Ser Cys Trp Leu
195 200 205

Cys Leu Ala Ser Gly Pro Pro Tyr Tyr Glu Gly Met Ala
210 215 220

<210> 8

<211> 203

<212> PRT

<213> porcine endogenous retrovirus Type PERV MSL

<400> 8

Asn Asn Gly Lys His Cys Gly Asn Pro Arg Asp Phe Phe Cys Lys Gln
1 5 10 15

Trp Asn Cys Val Thr Ser Asn Asp Gly Tyr Trp Lys Trp Pro Thr Ser
20 25 30

Gln Gln Asp Arg Val Ser Phe Ser Tyr Val Asn Thr Tyr Thr Ser Ser
35 40 45

Gly Gln Phe Asn Tyr Leu Thr Trp Ile Arg Thr Gly Ser Pro Lys Cys
50 55 60

Ser Pro Ser Asp Leu Asp Tyr Leu Lys Ile Ser Phe Thr Glu Lys Gly
65 70 75 80

Lys Gln Glu Asn Ile Leu Lys Trp Val Asn Gly Met Ser Trp Gly Met
85 90 95

Val Tyr Tyr Gly Gly Ser Gly Lys Gln Pro Gly Ser Ile Leu Thr Ile
100 105 110

Arg Leu Lys Ile Asn Gln Leu Glu Pro Pro Met Ala Ile Gly Pro Asn
115 120 125

Thr Val Leu Thr Gly Gln Arg Pro Pro Thr Gln Gly Pro Gly Pro Ser
130 135 140

Ser Asn Ile Thr Ser Gly Ser Asp Pro Thr Glu Ser Asn Ser Thr Thr
145 150 155 160

Lys Met Gly Ala Lys Leu Phe Ser Leu Ile Gln Gly Ala Phe Gln Ala
165 170 175

```
Leu Asn Ser Thr Thr Pro Glu Ala Thr Ser Ser Cys Trp Leu Cys Leu
            180                 185                 190

Ala Ser Gly Pro Pro Tyr Tyr Glu Gly Met Ala
        195                 200
```

<210> 9

<211> 203

<212> PRT

<213> porcine endogenous retrovirus Type Tsukuba

<400> 9

```
Asn Asn Gly Lys His Cys Gly Asn Pro Arg Asp Phe Phe Cys Lys Gln
1               5                   10                  15

Trp Asn Cys Val Thr Ser Asn Asp Gly Tyr Trp Lys Trp Pro Thr Ser
            20                  25                  30

Gln Gln Asp Arg Val Ser Phe Ser Tyr Val Asn Thr Tyr Thr Ser Ser
        35                  40                  45

Gly Gln Phe Asn Tyr Leu Thr Trp Ile Arg Thr Gly Ser Pro Lys Cys
    50                  55                  60

Ser Pro Ser Asp Leu Asp Tyr Leu Lys Ile Ser Phe Thr Glu Lys Gly
65                  70                  75                  80

Lys Gln Glu Asn Ile Leu Lys Trp Val Asn Gly Met Ser Trp Gly Met
                85                  90                  95

Val Tyr Tyr Gly Gly Ser Gly Lys Gln Pro Gly Ser Ile Leu Thr Ile
            100                 105                 110

Arg Leu Lys Ile Asn Gln Leu Glu Pro Pro Met Ala Ile Gly Pro Asn
        115                 120                 125

Thr Val Leu Thr Gly Gln Arg Pro Pro Thr Gln Gly Pro Gly Pro Ser
    130                 135                 140

Ser Asn Ile Thr Ser Gly Ser Asp Pro Thr Glu Ser Ser Ser Thr Thr
145                 150                 155                 160

Lys Met Gly Ala Lys Leu Phe Ser Leu Ile Gln Gly Ala Phe Gln Ala
                165                 170                 175

Leu Asn Ser Thr Thr Pro Glu Ala Thr Ser Ser Cys Trp Leu Cys Leu
            180                 185                 190

Ala Leu Gly Pro Pro Tyr Tyr Glu Gly Met Ala
        195                 200
```

<210> 10

<211> 221

<212> PRT

<213> porcine endogenous retrovirus Type PERV B

<400> 10

```
Glu Lys Glu Lys Tyr Cys Gly Gly Ser Gly Glu Ser Phe Cys Arg Arg
1               5                  10                  15

Trp Ser Cys Val Thr Ser Asn Asp Gly Asp Trp Lys Trp Pro Ile Ser
            20                  25                  30

Leu Gln Asp Arg Val Lys Phe Ser Phe Val Asn Ser Gly Pro Gly Lys
        35                  40                  45

Tyr Lys Val Met Lys Leu Tyr Lys Asp Lys Ser Cys Ser Pro Ser Asp
    50                  55                  60

Leu Asp Tyr Leu Lys Ile Ser Phe Thr Glu Lys Gly Lys Gln Glu Asn
65                  70                  75                  80

Ile Gln Lys Trp Ile Asn Gly Met Ser Trp Gly Ile Val Phe Tyr Lys
                85                  90                  95

Tyr Gly Gly Gly Ala Gly Ser Thr Leu Thr Ile Arg Leu Arg Ile Glu
            100                 105                 110

Thr Gly Thr Glu Pro Pro Val Ala Val Gly Pro Asp Lys Val Leu Ala
        115                 120                 125

Glu Gln Gly Pro Pro Ala Leu Glu Pro Pro His Asn Leu Pro Val Pro
    130                 135                 140

Gln Leu Thr Ser Leu Arg Pro Asp Ile Thr Gln Pro Pro Ser Asn Gly
145                 150                 155                 160

Thr Thr Gly Leu Ile Pro Thr Asn Thr Pro Arg Asn Ser Pro Gly Val
                165                 170                 175

Pro Val Lys Thr Gly Gln Arg Leu Phe Ser Leu Ile Gln Gly Ala Phe
            180                 185                 190

Gln Ala Ile Asn Ser Thr Asp Pro Asp Ala Thr Ser Ser Cys Trp Leu
        195                 200                 205

Cys Leu Ser Ser Gly Pro Pro Tyr Tyr Glu Gly Met Ala
    210                 215                 220
```

<210> 11

<211> 220

<212> PRT

<213> porcine endogenous retrovirus Type PoEV1

<400> 11

```
Glu Lys Glu Lys Tyr Cys Gly Gly Ser Gly Glu Ser Phe Cys Arg Arg
1               5                  10                  15

Trp Ser Cys Val Thr Ser Asn Asp Gly Asp Trp Lys Trp Pro Ile Ser
            20                  25                  30

Leu Gln Asp Arg Val Lys Phe Ser Phe Val Asn Ser Gly Pro Gly Lys
        35                  40                  45

Tyr Lys Met Met Lys Leu Tyr Lys Asp Lys Ser Cys Ser Pro Ser Asp
    50                  55                  60

Leu Asp Tyr Leu Lys Ile Ser Phe Thr Glu Arg Lys Thr Gly Lys Tyr
65                  70                  75                  80

Ser Lys Val Asp Lys Trp Tyr Glu Leu Gly Asn Ser Phe Leu Leu Tyr
                85                  90                  95

Gly Gly Gly Ala Gly Ser Thr Leu Thr Ile Arg Leu Arg Ile Glu Thr
            100                 105                 110

Gly Thr Glu Pro Pro Val Ala Met Gly Pro Asp Lys Val Leu Ala Glu
        115                 120                 125

Gln Gly Pro Pro Ala Leu Glu Pro Pro His Asn Leu Pro Val Pro Gln
    130                 135                 140

Leu Thr Ser Leu Arg Pro Asp Ile Thr Gln Pro Pro Ser Asn Ser Thr
145                 150                 155                 160

Thr Gly Leu Ile Pro Thr Asn Thr Pro Arg Asn Ser Pro Gly Val Pro
                165                 170                 175

Val Lys Thr Gly Gln Arg Leu Phe Ser Leu Ile Gln Gly Ala Phe Gln
            180                 185                 190

Ala Ile Asn Ser Thr Asp Pro Asp Ala Thr Ser Ser Cys Trp Leu Cys
        195                 200                 205

Leu Ser Ser Gly Pro Pro Tyr Tyr Glu Gly Met Ala
    210                 215                 220
```

<210> 12

<211> 15

<212> PRT

<213> porcine endogenous retrovirus

<400> 12

Arg Glu Glu Arg Arg Asp Arg Arg Gln Glu Lys Asn Leu Thr Lys
1 5 10 15

<210> 13

<211> 14

<212> PRT

<213> porcine endogenous retrovirus

<400> 13

Ala Arg Asn Cys Pro Lys Lys Gly Asn Lys Gly Pro Lys Val
1 5 10

<210> 14

<211> 16

<212> PRT

<213> porcine endogenous retrovirus Type PoEV1

<400> 14

Thr Ser Leu Arg Pro Asp Ile Thr Gln Pro Pro Ser Asn Ser Thr Thr
1 5 10 15

<210> 15

<211> 15

<212> PRT

<213> porcine endogenous retrovirus Type PERV B

<400> 15

Lys Gly Lys Gln Glu Asn Ile Gln Lys Trp Ile Asn Gly Met Ser
1 5 10 15

<210> 16

<211> 18

<212> PRT

<213> porcine endogenous retrovirus Type PoEV1

<400> 16

Arg Lys Thr Gly Lys Tyr Ser Lys Val Asp Lys Trp Tyr Glu Leu Gly
1   5   10   15

Asn Ser

<210> 17

<211> 12

<212> PRT

<213> porcine endogenous retrovirus Type Tsukuba

<400> 17

Asn Thr Val Leu Thr Gly Gln Arg Pro Pro Thr Gln
1   5   10

<210> 18

<211> 22

<212> PRT

<213> porcine endogenous retrovirus Type PERV A

<400> 18

Gly His Gly Arg Trp Lys Asp Trp Gln Gln Arg Val Gln Lys Asp Val
1   5   10   15

Arg Asn Lys Gln Ile Ser
            20

<210> 19

<211> 16

<212> PRT

<213> porcine endogenous retrovirus Type PERV A

<400> 19

Ile Gln Glu Gln Arg Pro Ser Pro Asn Pro Ser Asp Tyr Asn Thr Thr
1   5   10   15